THE EFFECT OF OXIDIZING AGENTS ON CERTAIN NON-SPORE-FORMING ANAEROBES

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INTRODUCTION

Oxidizing agents are commonly used in the treatment of infections in which anaerobic organisms are suspected of being the etiological agent. The usual reason advanced for their use is that anaerobes are intolerant of oxygen, and therefore, a chemical liberating oxygen should be a specific remedy. Little or no consideration is given to the question of why anaerobes are intolerant of oxygen, or to the extent of their intolerance.

Stephenson (1939) states that the sensitivity of certain anaerobes to oxygen still awaits satisfactory explanation. The characteristic intolerance of anaerobes to oxygen might be explained by their tendency to produce hydrogen peroxide when brought in contact with air, as it can be shown that their growth is inhibited by the presence of traces of peroxide in media incubated in an atmosphere free from oxygen. Being devoid of catalase, they are unable to protect themselves from the hydrogen peroxide formed (McLeod, 1930).

The harmful effects of peroxide on bacteria are probably not due to their tendency to liberate molecular oxygen (Gifford, 1888; McLeod and Gordon, 1923; and Hewitt, 1936).

It is quite probable that anaerobiosis is a matter of oxidation-reduction potential rather than simple absence of oxygen (Dack and Burrows, 1935). It is not, however, possible in general to define a particular oxygen tension which will limit the growth of an anaerobe (Vennesland and Hanke, 1940).

Vennesland and Hanke (1940) found that an actively growing culture of *Bacteroides vulgatus*, an obligate anaerobe, can tolerate a temporary overdose of oxygen accompanied by a considerable rise in potential without apparent ill effect. If, however, the air treatment is continued for a longer period of time (10 to 20 hours) actual injury may be sustained. The potential measured in the *Bacteroides vul-*

gatus cultures indicates the state of a reversible oxidation-reduction system. Oxygen affects the potential by oxidizing this system (directly or indirectly). The oxidizing action of oxygen is counteracted by reducing substances present in the culture which tend to reduce the reversible system. Apparently oxygen tension controls the growth of this organism only in so far as it influences the potential (Vennesland and Hanke, 1940).

Anaerobes are unable to effect any reduction in ordinary aerobic cultures. If, however, the medium is reduced at the start, they are able to establish more intensive reducing conditions (Hewitt, 1936).

Preliminary reduction of media may be affected by various methods: 1. Hydrogen in an anaerobic jar. 2. Pure nitrogen. 3. Various chemical reducing agents. 4. Growth of an aerobic organism.

The reducing effect of —SH compounds is important in anaerobiosis. *Clostridium sporogenes*, for example, after exposure to oxygen or dilute hydrogen peroxide exhibits a long latent period in its anaerobic growth. This latent period is diminished or almost entirely removed in the presence of cystine or other —SH compounds in the medium (Quastel and Stephenson, 1926). If the velocity of oxidation of the —SH is greater than the rate of its formation, then proliferation cannot occur but if proliferation has already commenced by reason of initial presence of a quantity of —SH, then the proliferation will continue for as long as the minimal concentration of —SH is maintained (Vennesland and Hanke, 1940).

It is well known that fresh tissues are capable of inducing growth of anaerobes in broth. However, attempts to obtain surface growth of obligate anaerobes in pure culture in contact with air have not been successful (Hall, 1928).

We cannot exclude the possibility of a direct oxidizing action of "active oxygen" on the protoplasm of the bacterial cell or a direct combination of oxygen with cellular enzymes. At the present time, however, there is no experimental data to prove such assumptions (Johnson and Meleney, 1939).

EXPERIMENTAL

Atmospheric oxygen is definitely bacteriostatic for anaerobes but its immediate bacteriocidal effect is doubtful. Furthermore, there is no logical reason for assuming that obligate anaerobes are any less susceptible to the action of commonly used antiseptics and

germicides than are aerobic organisms. Experiments were designed, therefore, to: (A) compare the immediate germicidal effect of oxidizing agents on obligate anaerobes with the germicidal effect of the more commonly used chemicals. (B) compare the germicidal effect of oxidizing agents on anaerobes with their effect on common aerobes. (C) compare the germicidal effect of oxidizing agents and other chemicals on anaerobes in the presence of blood. (D) test the prolonged effect of oxidizing agents on anaerobes in the presence of blood. (E) test the protective effect of blood on anaerobes exposed to atmospheric oxygen.

The anaerobic organisms used in these experiments were pure cultures of Gram-negative fusiform-like bacteria isolated from the oral cavities of two individuals. They were morphologically and culturally similar to organisms described as *Fusiformis nucleatus* by Hine and Berry (1937). The chief difference in our two strains which are designated fusiform bacillus (A) and fusiform bacillus (B) was that the latter was the more fastidious in its growth requirements.

Aerobic organisms tested were laboratory strains of *Escherichia coli*, *Eberthella typhosa*, *Staphylococcus aureus* and *Staphylococcus albus*.

Stock cultures of anaerobes were maintained in tubes of cooked meat medium (Kolmer and Boerner, 1938) and transplanted every two weeks. Stock cultures of aerobes were maintained on meat extract agar and transplanted every month. After growth was definitely established, the anaerobes were stored at room temperature and the aerobes at 8°C.

Numerous preliminary experiments established the following points: 1. Forty-eight hour anaerobic cultures of the fusiform-like organisms on blood agar plates or in deep broth suspensions were found to remain viable for several hours when exposed to atmospheric oxygen. 2. Douglas broth (Zinsser-Bayne Jones, 1939) was found to be a very efficient medium for the cultivation of the fusiform bacilli. 3. Results of germicidal tests performed according to the standard technics for determining the phenol coefficient were inconsistent.

Several experiments were made and are described separately, but the following general technic was employed throughout.

Test cultures were made by inoculating 100 cc. of Douglas broth with 0.5 cc. of the stock culture in case of anaerobes, and with a 2 mm. loopful of growth from an agar slant in case of aerobes. Forty-eight hour anaerobic and 18 hour aerobic cultures were used.

The anaerobic technic was similar to the McIntosh-Fildes method (Fildes, 1931). The jars were evacuated to 70 to 75 mm. of mercury and then hydrogen was allowed to flow in slowly for 40 to 45 minutes. An electrically heated platinized asbestos coil was used as the catalyst. In each jar there was a slight positive hydrogen pressure.

In order to minimize the sampling error 25 per cent or 50 per cent of the culture was included in the test mixture of the organisms and chemicals.

Transplants were inoculated by making 1:10 serial dilutions in broth of test mixtures. Anaerobes were transplanted to Douglas broth and incubated for 5 days. Aerobes were transplanted to peptone water and incubated for 4 days. Positive growth was indicated by turbidity and checked by plating and studying the resultant growth.

Chemicals for comparison with oxidizing agents were chosen because of their common usage, and because their bacteriostatic effect does not greatly exceed their bacteriocidal effect. Stock chemicals employed were hydrogen peroxide 30 per cent (C.P. Merck), sodium perborate (U.S.P. Merck), potassium permanganate, crystals (C.P. Merck), iodine crystals (C.P. Baker), phenol (C.P. Baker) and zinc peroxide (Merck Special-

Medicinal Z.P.O.). The zinc peroxide was heated at 140°C for 4 hours before use, according to the recommendation of Johnson and Meleney (1939). Distilled water containing 0.85 per cent NaCl was used as the diluent in all cases. In case of iodine, 3 per cent KI was used to make the one per cent stock solutions.

It is realized that tests for the evaluation of antiseptics and germicides are subject to considerable variation (Knighton, 1939). Therefore, all experiments were performed in triplicate. The recorded results are those which were constant in three tests.

In several cases the difference between the killing dilution and the concentration permitting growth was relatively wide. For example, a 1:50 to 1:100 dilution for hydrogen peroxide and 1:1000 to 1:1500 for iodine. The tests in such cases were not sufficiently sensitive to obtain constant results in a closer range. However, the results do have a comparative value.

Experiment # 1. A comparison of the relative germicidal effect on anaerobes and aerobes of oxidizing agents and other commonly used chemicals.

Mechanism of the test: Two cc. of broth culture were mixed with an equal volume of the designated dilution of the chemical in a ½" by 6" test tube. Both chemicals and culture were at room temperature. After mixing they were placed in a 37°C. water bath. At the end of 5 minutes transplants was made by pipetting 0.5 cc. of the mixture to 5 cc. of Douglas broth, in the case of anaerobes, or to 5 cc. of peptone water in the case of aerobes. After mixing well, 0.5 cc. of this mixture was transferred to a second 5 cc. of the corresponding broth. The second tube containing an approximately 1:100 dilution of the test mixture was incubated as previously described. The first transfer tubes contained 5 per cent of blood throughout this series of tests in order to counteract the hydrogen peroxide carried over.

Control tests revealed that the amount of chemicals carried over into the transfer tubes was not sufficient to exert a significant bacteriostatic effect. The results are recorded in Table I.

A comparison of the bacteriocidal effects of the chemicals indicated that: 1. Hydrogen peroxide was the most effective of the oxygen liberating compounds. 2. Phenol had about the same effect on the anaerobes as did hydrogen peroxide. 3. Iodine was far more effective against the anaerobes than was hydrogen peroxide. 4. The amount of potassium permanganate required was beyond its practical range. 5. Hydrogen peroxide and sodium perborate were more effective against anaerobes than against aerobes. 6. Phenol and iodine were as effective against anaerobes as against aerobes.

Experiment # 2. To test the effect of whole blood on the immediate bacteriocidal action of chemicals on anaerobes.

Mechanism of the test: Two cc. of broth culture, 1 cc. of defibrinated rabbit blood and 1 cc. of twice the designated dilution of the chemical were mixed in a ½" x 6" test tube.

Twice the designated dilution was used to allow for the blood and still have a dilution of the chemical comparable to that used in Experiment #1.

After 5 minutes in a 37°C. water bath, transfers were made as described in Experiment #1 except that the 5 per cent blood was not included in the first transfer tubes since blood was present in the original mixtures. The results are recorded in Table II.

TABLE I

Comparative effect of chemicals on anaerobic and aerobic organisms

TEST ORGANISMS	CHEMICALS TESTED									
	Hydrogen peroxide		Sodium perborate		Potassium permanganate		Iodine		Phenol	
	I	II	I	II	I	II	I	II	I	II
Fusiform bacillus (A)	1:50	1:100	1:40	1:50	1:60	1:70	1:1000	1:1500	1:70	1:80
Fusiform bacillus (B)	1:100	1:200	1:60	1:80	1:70	1:100	1:1000	1:1500	1:90	1:100
Escherichia coli	3:100	1:50	1:20	1:40			1:1000	1:1500	1:60	1:80
Eberthella typhosa	3:100	1:50	1:20	1:40			1:1000	1:1500	1:80	1:100
Staphylococcus aureus	1:10	1:25		1:20			1:500	1:1500	1:40	1:60
Staphylococcus albus	2:25	3:50		1:20			1:500	1:1000	1:40	1:60

Column I under each chemical denotes the dilution always killing the test organisms in 5 minutes under conditions of this test.

Column II denotes the concentration always allowing growth under the same conditions.

TABLE II

Comparative effect of chemicals on anaerobes in the presence of blood

ORGANISMS	CHEMICALS*							
	Hydrogen peroxide		Sodium perborate		Iodine		Phenol	
	I	II	I	II	I	II	I	II
Fusiform bacillus (A)		15:100		1:40	1:200	1:300	1:60	1:70
Fusiform bacillus (B)		15:100		1:40	1:200	1:400	1:60	1:70

Column I under each chemical denotes dilution constantly killing the test organisms in 5 minutes.

Column II denotes the concentration constantly allowing growth after 5 minutes exposure.

* Each test mixture contained 25 per cent defibrinated rabbit blood.

In the presence of whole blood a 2.5 per cent solution of sodium perborate and a 15 per cent solution of hydrogen peroxide failed to kill the anaerobes in 5 minutes according to the conditions of this test.

Iodine in a 1:200 dilution and phenol in a 1:60 dilution were effective in killing the organisms in 5 minutes. Therefore, iodine and phenol appeared to be far more effective against the anaerobic bacteria in the presence of blood than hydrogen peroxide and sodium perborate.

Experiment #3. To test the prolonged effect of hydrogen peroxide, sodium perborate and zinc peroxide on anaerobes in the presence of blood.

Mechanism of the test: Two cc. of the broth culture, two cc. of defibrinated rabbit blood and four cc. of the designated dilution of the chemical were mixed in 1″ x 8½″ test tubes.

TABLE III

The prolonged effect of oxidizing agents on anaerobes in the presence of blood

FUSIFORM BACILLUS (A) 1 PART, BLOOD 1 PART, AND 2 PARTS OF:	HIGHEST 1:10 DILUTION OF TEST MIXTURES IN DOUGLAS BROTH SHOWING GROWTH AFTER DESIGNATED EXPOSURE				
	5 Mins.	2 Hrs.	4 Hrs.	6 Hrs.	18–20 Hrs.
30% Hydrogen peroxide mixed in flask		10^{-5}			10^{-4}
30% Hydrogen peroxide mixed in tubes		10^{-7}			10^{-5}
2.5% Sodium perborate	10^{-6}	10^{-5}	10^{-4}	10^{-3}	—
5% Zinc peroxide	10^{-5}		10^{-3}	10^{-3}	—
N/20 Sodium hydroxide	10^{-6}	10^{-5}			—
0.85% Sodium chloride	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}

— = No growth.

In an additional test, 4 cc. of culture, 4 cc. of blood and 8 cc. of hydrogen peroxide were mixed in a 500 cc. Erlenmeyer flask. After 2 hours the liquid settling from the immediate bubbling was pipetted into a ⅝″ x 6″ test tube.

The mixtures were placed in a 37°C. water bath and 1:10 serial dilutions made at desired intervals by pipetting 0.5 cc. into a test tube containing 4.5 cc. of Douglas broth. Individual pipettes were used for each dilution. Saline (0.85 per cent) and N/20 NaOH were used as control tests. Results are recorded in Table III.

This test indicates only the relative number of viable organisms at different intervals as compared with the 0.85 per cent NaCl controls. The viable counts, according to these results, were relatively constant in the 0.85 per cent NaCl controls up to 20 hours.

Tests with hydrogen peroxide in the tubes indicated very little drop in viable organisms after 2 hours. There was a greater drop in flask mixtures but this is of questionable significance since the

heat produced by these mixtures might account for some of the reduction. There was a definite reduction after 20 hours but the number of viable organisms remaining was still significant.

The comparison of these results with those in Table I, where hydrogen peroxide was used without catalase, apparently supports the view that hydrogen peroxide was far more effective, as such, than was the oxygen liberated.

The results of the experiments with sodium perborate indicated that: (a) the drug cannot be depended upon for an immediate destruction of the organisms in the presence of blood. (b) There was a progressive lowering of the viable count with complete destruction within 20 hours.

Why, then, was a 1:40 dilution of sodium perborate effective as recorded in Table I? This immediate effect in the absence of catalase may be due to the hydrogen peroxide formed in the solution. After dissolving in water 25 per cent of the sodium perborate content was found to be hydrogen peroxide. This would indicate that the 1:40 dilution contained approximately a 1:160 dilution of hydrogen peroxide. This was weaker than the 1:50 to 1:100 dilution of hydrogen peroxide required, but the difference might be explained by the combined effect of hydrogen peroxide and sodium compounds.

The prolonged effect of sodium perborate was hardly due to its prolonged oxidizing effect in view of the great amount of catalase present. It is more reasonable to suspect other factors. The NaOH formed or the high alkalinity (approximately pH 9.5 to 10) may be responsible. This assumption receives some support in the fact that N/20 NaOH mixtures gave approximately the same pH and bacteriocidal effect. Possibly, there are other sodium compounds which might account for its action.

Five per cent zinc peroxide gave approximately the same results as a 1:40 dilution of sodium perborate. This action may be due to small amounts of hydrogen peroxide formed in addition to the continued liberation of oxygen as suggested by Johnson and Meleney (1939). It seems illogical, however, to expect hydrogen peroxide to accumulate even in small amounts in the presence of so much catalase. Its action might be more logically attributed to the effect of continued oxidation of the oxidation reduction system. Then as suggested by

Garrod (1940) ZnO, $ZnCO_3$ and $Zn(OH)_2$ compounds may be present and contribute to the prolonged effect.

Experiment # 4. To test the effect of a constant amount of hydrogen peroxide on anaerobes in varying amounts of blood.

Mechanism of the test: Two cc. of broth culture, two cc. of blood and four cc. of three per cent hydrogen peroxide were mixed in 1.0" x 8½" test tubes. The mixtures were placed in the 37°C. water bath and 1:10 serial dilutions in Douglas broth were made as described in Experiment # 3. The results of Experiment # 4 are recorded in Table IV.

If 5 per cent or 10 per cent of blood were included in the suspensions, the number of viable organisms was not significantly reduced. If only 1 per cent of blood was included, there was a relatively significant drop in viable organisms within 5 minutes and a very significant drop within 2 hours. There were no viable organisms at the end of 20 hours. The immediate effect of 3 per cent hydrogen peroxide was apparently not inhibited by inclusion of only 0.25 per cent of blood.

TABLE IV

The prolonged effect of hydrogen peroxide on anaerobes in the presence of varying amounts of blood

3% HYDROGEN PEROXIDE 2 PARTS, FUSIFORM BACILLUS (A) 1 PART, AND 1 PART OF:	HIGHEST 1:10 DILUTION IN DOUGLAS BROTH OF THE TEST MIXTURES SHOWING GROWTH AFTER DESIGNATED EXPOSURES		
	5 Mins.	2 Hrs.	18-20 Hrs.
40% blood		10^{-7}	10^{-7}
20% blood	10^{-6}	10^{-6}	10^{-6}
4% blood	10^{-4}	10^{-2}	—
1% blood	—		

— = No growth.

This is apparently further evidence that hydrogen peroxide as such was the immediate bacteriocidal factor instead of liberated oxygen.

Experiment # 5. To test the protective effect of small amounts of blood on the "autosterilizing" effect of aerated cultures.

Mechanism of the test: Small amounts of broth culture with and without blood and ascitic fluid were placed in 250 cc. Erlenmeyer flasks as follows: Flask # 1, 1.5 cc. of culture. Flask # 2, 1.5 cc. of culture containing 1 per cent blood. Flask # 3, 1.5 cc. of culture containing 1 per cent ascitic fluid. Flask # 4, 1.5 cc. of culture containing 5 per cent ascitic fluid. These "shallow layers" were placed in a 37°C. incubator under aerobic

conditions for 24 hours. Serial dilutions in Douglas broth were then made as described in Experiment #3.

A growth was obtained through a 10^{-4} dilution in those dilutions made from Flask #2 containing 1 per cent blood. No growth in any dilution was obtained from the other flasks.

There was a definite autosterilizing effect within 24 hours in shallow layers of this culture under aerobic conditions.

The inclusion of 1 per cent of defibrinated blood prevented a complete destruction of the organisms under these conditions. Ascitic fluid, 1 to 5 per cent, did not exert this protective effect. This could be explained on the basis that the blood was protective due to the catalase which prevented an accumulation of hydrogen peroxide. Other possibilities are that the blood adds nutritive properties, collodial protection, or oxygen reducing properties. Since 5 per cent of ascitic fluid did not protect the culture to the same extent, the evidence seems to be more in favor of catalase as the chief protective factor.

The results indicated that small amounts of blood may exert a protective effect on anaerobes exposed to atmospheric oxygen.

GENERAL DISCUSSION

This paper deals chiefly with the question of (a) the action of oxidizing agents on bacteria; (b) a comparison of the effect on anaerobes of oxidizing agents and other chemicals. No attempt has been made to discuss two controversial questions that this report will probably suggest namely, (a) the significance of anaerobes in oral lesions as Vincent's infection. (b) The value of antiseptics and germicides in wound healing. For a noteworthy consideration of these subjects the reader is referred to the reports of Anderson (1938, Lyons (1938 and 1940), Rosebury (1938) and Appleton (1933).

However, it is evident that the destruction of anaerobic bacteria in tissues and wounds is a far more complicated procedure than the mere flash exposure to oxygen liberated by oxidizing agents. This is due to the catalase present and the reducing properties of blood and tissues, and to the symbiotic effect of other bacteria.

The only logical reasons for depending upon certain oxidizing agents are: (a) for a mechanical cleansing effect, or (b) for the long continued use of a relatively nontoxic substance.

It is realized that numerous clinical reports may be cited to show the definite value of hydrogen peroxide and sodium perborate in treating oral infections. They undoubtedly are helpful in many cases but it is entirely possible that their beneficial effect is due to mechanical cleansing rather than to the oxidizing effect on anaerobes.

In the presence of blood, the immediate bacteriocidal effect of sodium perborate is doubtful. The prolonged effect exerted by this compound is apparently due to factors other than the liberation of oxygen. Any possible toxic action (Council on Dental Therapeutics 1935) (Miller, Sorrin, Greenhut and Pelzer, 1938) of the drug would contraindicate its prolonged application.

Zinc peroxide offers the most promising results as a prolonged oxidizing agent. Apparently the experiments of Meleney and Harvey (1939) and Johnson and Meleney (1939) definitely establish the value of this compound in controlling certain infections due to anaerobic and micro-aerophilic organisms.

The value of zinc peroxide depends on the long continued close contact with the tissues (Meleney and Harvey, 1939). This fact is a definite drawback to its use in the oral cavity.

SUMMARY

Hydrogen peroxide, as such, is far more bacteriocidal for anaerobes than is the oxygen liberated.

Anaerobic bacteria may tolerate a temporary exposure to oxygen liberated by oxidizing agents without being destroyed. The germicidal effect of sodium perborate on anaerobes is probably due to factors other than mere liberation of oxygen. These factors may also exert a deleterious effect on tissues.

Zinc peroxide offers the most promising results as a prolonged oxidizing agent.

The difficulty of keeping the zinc peroxide in contact with oral tissues for prolonged periods must be considered.

There is no reason to assume that anaerobes are any less susceptible to the germicidal action of commonly used chemicals than are aerobes.

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TRAUMATIC CHANGES IN THE PERIODONTAL TISSUES OF DECIDUOUS TEETH

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The morphology of traumatic changes in the supporting tissues of human teeth has been known for only a short time. It is little more than ten years since the first specimens of this kind were reported and correctly interpreted. The experiments of Gottlieb and Orban (1) with the teeth of animals greatly assisted in evaluating the significance of the comparable findings in human teeth. Coolidge (2) reviewed the literature on this subject, in conjunction with a description of additional findings; therefore, it seems unnecessary at this time to review again the now rather voluminous literature.

Almost all of the findings on traumatic changes in the human periodontal tissues have been from the teeth of adults, with the exception of findings reported by Oppenheim (3), Bauer (4), Gottlieb and Orban (1) and Coolidge (2), who observed similar traumatic injuries in the deciduous teeth of children with mixed dentitions. Therefore, it seemed justified to make a systematic examination of the deciduous dentitions of younger children and infants, covering the period from birth to the time of shedding.

The material examined for this study consisted of 6 complete human jaws of the following ages: 9 months, 15 months (?), $1\frac{1}{2}$ years, 2 years, 3 years, and $4\frac{1}{2}$ years. Evidence of traumatic injuries were found in all of them.

HISTOLOGIC FINDINGS

Jaw, Protocol # 13. Female, aged 9 months, cause of death enteritis.

In this jaw the only teeth that have erupted are the mandibular deciduous first incisors. On the left incisor only the incisal edge is visible; on the right incisor less than half of the anatomical crown has

[1] Deceased.

erupted (*fig. 1*). The bottom of the gingival crevice on the labial and lingual sides of this crown is located at about the middle of the length of the crown. The root end is wide open and the growth of the root is still incomplete. The position of this tooth in its alveolus indicates that a force has been exerted upon the crown which tilted the latter

FIG. 1. Labiolingual section through an erupting mandibular deciduous first incisor. Age, 9 months. Root end deviates toward labial side of alveolus, causing compression of the periodontal membrane in area marked by square. E, enamel; D, dentin; P, pulp; G, germ of permanent central incisor. Magnification (× 10).

toward the lingual and the root end towards the labial side. This is indicated by the fact that the periodontal space is very wide at the labial alveolar margin and on the lingual side of the root end and that on the labial side of the root end, the root surface was forced against the bony wall, producing compression of the periodontal soft tissue in this area (*fig. 2*). The spicule of bone marked B^1 represents a

remnant of an older alveolar wall, perhaps of the original bone crypt, whereas the present alveolar wall is indicated by the surface of the bone, B.

The injured connective tissue between bone, B^1, and the root surface stains poorly and shows evidence of beginning hyalinization. An early stage of undermining resorption of the bone in this area of com-

FIG. 2. Higher magnification of area marked by square in *fig. 1*. P, pulp; PD, predentin; D, dentin; PM, periodontal membrane; B^1, isolated spicule of bone; OC osteoclast; B, alveolar wall. At X, periodontal soft tissues are compressed; nuclei are not stained, indicating hyalinization of damaged tissues. Magnification ($\times \pm 100$).

pression is indicated by the presence of osteoclasts (OC) on the surface of the bone spicules away from the tooth surface. This reaction is characteristic of traumatic periodontal injuries and signifies an attempt on the part of the tissues in the vicinity to remove the damaged structures (5). These changes may be explained in the following manner: Before the eruption of the deciduous teeth begins, the

jaw ridges meet during occlusion. As the first teeth, the mandibular first incisors, appear in the mouth, they interfere with the occlusion and are therefore subjected to considerable stress when the jaws are firmly closed. On the other hand, the periodontal membrane of such erupting teeth is not yet adapted to support occlusal stress. As a result of these two conditions, the tooth may easily be displaced in its socket, and traumatic injuries may occur at the points of greatest compression of the periodontal membrane.

FIG. 3. Bifurcation of mandibular right first deciduous molar. Age, about 15 months. Traumatic destruction of periodontal membrane at points where root surface is forced into contact with bone spicules. D, dentin; C, cementum; AB, spicules of alveolar bone; N, areas of hyalinization and necrosis of periodontal membrane; CY, hemorrhagic cyst; OC, osteoclasts. Magnification (× 50).

Jaw, Protocol #7. No data available, exact age unknown, state of development and eruption correspond to about 15 months.

In this jaw the 8 deciduous incisors and the 4 deciduous first molars are visible; apparently the latter have just come through the mucosa. In the bifurcation of all 4 deciduous first molars there have been extensive traumatic injuries with necrosis of the periodontal soft tissues and formation of cavities (*fig. 3*) containing structureless hemorrhagic material discussed in conjunction with *fig. 9*. The bone trabeculae in this area are densely covered with osteoclasts, indicating active

undermining resorption. It appears that in this stage of eruption the main masticatory load was borne by the first deciduous molars.

Jaw, Protocol # 14. Female, aged $1\frac{1}{2}$ years, cause of death—meningitis.

In this jaw all deciduous teeth are visible except the deciduous second molars. Of the deciduous canines and deciduous first molars less than one-half of the anatomical crown is erupted. Traumatic changes are found in several teeth, particularly in the 4 erupting

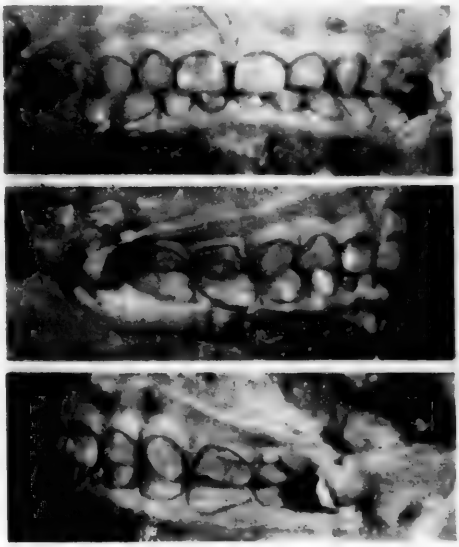

FIG. 4. Anterior and right and left side views of jaw, Protocol # 16. Age, 2 years. Complete deciduous dentition in good occlusion.

deciduous molars. Both at the root ends and in the bifurcations, the periodontal membrane has been compressed and undermining resorption has started. There are also similar changes in the deciduous first incisors. In these teeth a portion of the alveolar wall has been resorbed by the growing permanent tooth germs.

Jaw, Protocol # 16. Female, aged 2 years, cause of death—endocarditis.

Fig. 4 shows views of the deciduous dentition from the anterior and from the right and left sides. All deciduous teeth are present

and in normal occlusion; the teeth are intact except for some incipient caries in the maxillary right deciduous molars. The jaws were photographed and radiographed; then the left side of both the upper and lower jaw was sectioned buccolingually, the right lower jaw mesiodistally, and the right upper jaw horizontally. Traumatic changes were found in the following teeth:

IV I	I II III
V IV	IV V

As an example of the conditions in this jaw, the mandibular right deciduous molar is illustrated; the findings in the other teeth of this

FIG. 5. Radiograph of right posterior portion of mandible, Protocol #16 (*Fig. 4*). IV, deciduous first molar; V, deciduous second molar; 6, permanent first molar; 7, crypt of the permanent second molar, which appears empty.

jaw are very similar. *Fig. 5* is a radiograph of the right side of the mandible. The germ of the first premolar is situated between the roots of the deciduous first molar; the germ of the second premolar is not yet visible radiographically. The crown of the permanent first molar is nearing completion; behind and above this tooth can be seen the radiolucent crypt of the permanent second molar. The same area is shown in *fig. 6*, a photomicrograph of a mesiodistal section. The histologic picture corresponds closely to the radiograph. The germ of the second premolar is not visible since it is still located on the lingual side of the deciduous second molar, where it was orig-

inally formed. The permanent second molar can be seen in its crypt; its calcification has not yet started.

The deciduous second molar is still actively erupting. There are several indications of this: its cemento-enamel junction is still below that of the adjoining first deciduous molar, so that the transeptal fibres run obliquely between the two teeth; the alveolar crest of the deciduous second molar is at a deeper level than that of the deciduous first molar; and the trabeculae around both roots of the deciduous second molar are directed toward the occlusal plane, an arrangement which is indicative of an active occlusal movement of this tooth.

Fig. 7 shows the end of the distal root of the deciduous second molar. The apex is not yet completed. Directly above the apical opening on the mesial side there is an area of active root resorption, R_1, which extends well into the dentin; numerous osteoclasts are seen on the exposed dentin surface. Higher up, nearer the bifurcation on the same side, is another resorption area, R_2. Here the resorptive process is no longer active; there has been a reparative deposition of cementum. In the periodontal membrane there is evidence of a former traumatic injury due to compression of the type illustrated in *figs. 2* and *3*; however, at the time of death most of this compressed area has been removed by undermining resorption. In *fig. 8* the mesial root of the same tooth is reproduced. Again there are multiple areas of resorption on the distal aspect of the mesial root, all of which have been repaired by the deposition of cementum. The vertical arrangement of the bone trabeculae in the direction of eruption, mentioned in the description of *fig. 6*, is quite evident.

In the bifurcation of the deciduous second molar the thickness of the periodontal membrane is markedly decreased; in several places the periodontal soft tissue is compressed between cementum and bone, causing hyalinization and necrosis of the periodontal membrane and thrombosis of the periodontal capillaries. A similar condition is found in the bifurcation of the deciduous first molar; however, here the injury is apparently of longer standing, for there has been considerable undermining resorption of the bone in the damaged areas. The distal root of this tooth shows areas of resorption comparable in extent and location to those illustrated in *figs. 7* and *8*.

FIG. 6. Mesiodistal section through area shown in the radiograph in *fig. 5*. IV, deciduous first molar; 4, germ of first premolar; V, deciduous second molar; 6, germ of permanent first molar; 7, germ of permanent second molar; MC, mandibular canal; TS, transeptal fibers between deciduous first and second molars. Magnification (× 5).

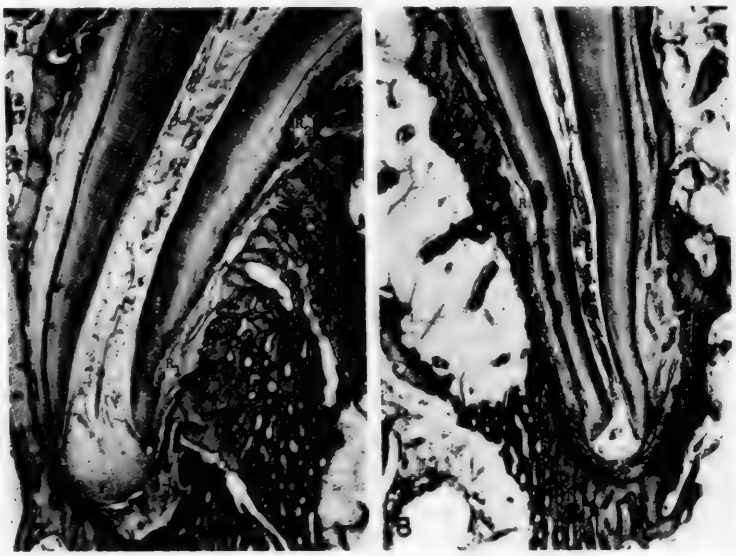

FIG. 7. Distal root of deciduous second molar shown in *fig. 6*. R_1, active root resorption near apex; R_2, repaired root resorption near bifurcation; T, trabeculae of alveolar bone arranged in the direction of eruption. Magnification (× ±18).

FIG. 8. Mesial root of deciduous second molar shown in *fig. 6*. R, repaired resorptions on under surface of root; T, bone trabeculae arranged in direction of eruption. Magnification (× ±18).

Jaw, Protocol # 15. Female, aged 3 years, cause of death—miliary tuberculosis.

In this jaw all deciduous teeth are present and in occlusion. There are hypoplastic defects and early stages of caries in the maxillary deciduous first and second incisors and in the deciduous first molars. There is also some evidence of decay in the occlusal fissures

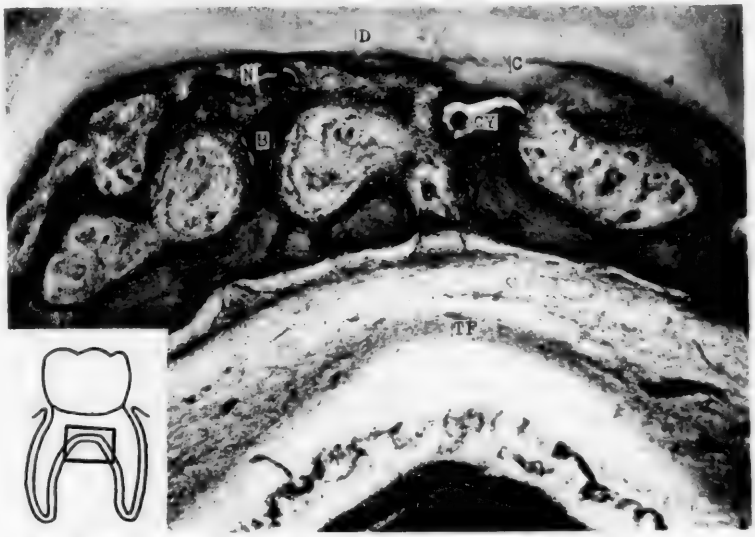

FIG. 9. Bifurcation of mandibular right deciduous second molar. Jaw, Protocol #15; age, 3 years. Traumatic destruction of periodontal membrane at points where tooth surface comes in contact with bone trabeculae. D, dentin; C, cementum; N, necrosis of the periodontal membrane; B, bone trabeculae arranged in direction of eruption; CY, hemorrhagic cyst; TF, tooth follicle of permanent tooth germ. Magnification (× ±33).

of the deciduous second molars. The occlusion was practically normal.

Histologic evidence of traumatic changes are found in the following teeth:

V IV	III V
V IV	I IV V

The findings are quite uniform, the only variation being in the intensity of the damage, which ranges from resorption of the alveolar wall,

to the formation of hemorrhagic cysts. The latter condition is illustrated in *fig. 9*. In this area, the bifurcation of the mandibular right deciduous second molar, the root surface is in contact with the upper ends of several bone trabeculae which are arranged in the general direction of eruption. In the same places uncalcified young bone can still be seen at the ends of the trabeculae. Apparently the tooth was pressed forcibly against this bone, for the soft tissue between bone and cementum has been crushed, and small hemorrhages from the damaged periodontal capillaries have occurred. Some of these areas were walled off, thus producing what has been termed a hemorrhagic cyst. Such cavities of varying size are found in the body wherever old hemorrhages have been resorbed, leaving behind cavities containing a brownish fluid or structureless brownish particles. They are not true cysts, since they lack an epithelial lining, but are comparable, for instance, to the cavities found in the brain as a result of an old cerebral hemorrhage. In the periodontal membrane they were first described by Bauer (4).

In several places in this jaw there is evidence of recurrence of these traumatic injuries. *Fig. 10* shows an area in the bifurcation of the mandibular right deciduous first molar. Resorption has taken place on the root surface, caused apparently by earlier trauma. The presence of an intact bony wall between deciduous and permanent tooth makes it very improbable that these resorptions could have been caused by the pressure of the growing tooth germ. They were later repaired by the deposition of cementum. Shortly before death, bone and root surface came again in contact. The periodontal membrane was crushed in several areas, and the bone was removed by undermining resorption.

Jaw, Protocol #8. Female, aged $4\frac{1}{2}$ years, cause of death not known.

In this jaw all deciduous teeth are erupted and in normal occlusion. Caries is present in all maxillary and mandibular deciduous molars. The maxillary and mandibular anterior teeth are widely spaced.

Traumatic injuries are found in the following teeth:

V IV III I
V IV III I I IV V

The injuries are of the same nature as those shown in *figs. 7* to *10*; therefore, they will not be illustrated again. In addition to the traumatic changes, there are resorptions on the roots of these teeth which

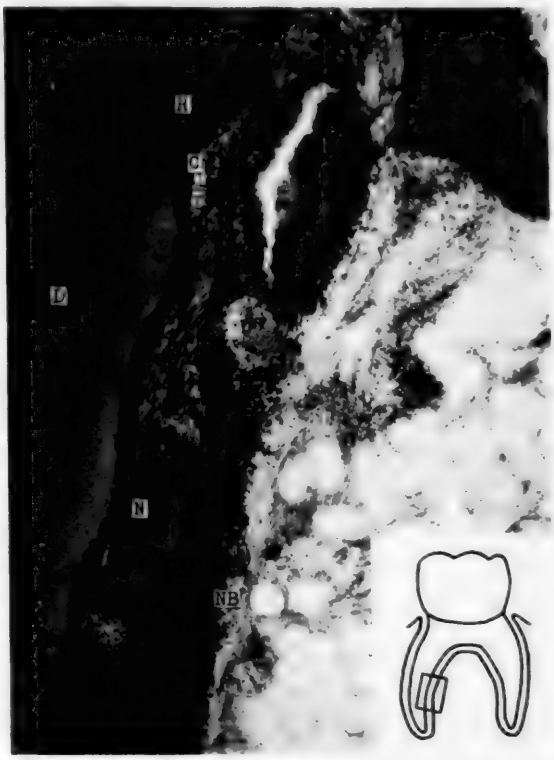

FIG. 10. Traumatic injury in previously resorbed and repaired area of root surface. Mandibular right deciduous first molar. Jaw, Protocol #15; age, 3 years. D, dentin; R, extent of previous resorption; C, new cementum deposited on resorbed dentin surface; N, necrotic periodontal membrane between cementum and bone; NB, formation of new bone. Magnification (# ±64).

were caused by the growth and movement of the permanent tooth germs.

COMMENTS

In the six jaws of infants reported in this study, traumatic tissue injuries are found in more than one-half of all erupted deciduous

teeth. In view of the fact that only a limited number of sections of each tooth is available, and that each tooth has been sectioned in only one plane, it is reasonable to assume that in reality the incidence of such injuries is still higher.

Heretofore, only two factors have been considered in the etiology of such changes. One is the intrinsic factor, namely, the weakening of the periodontal support. In the case of deciduous teeth, such weakening may be due to resorption of bone and root (shedding), and in the case of permanent teeth, to bone resorption of any kind. The other factor is extrinsic, namely, excessive occlusal stress, produced by malalignment, mutilation, loss or extraction of teeth, or by dental restorations.

In regard to the changes described in this report, especially in the jaws of less than three years of age, there is not sufficient evidence that it is the effect of shedding which caused the described traumatic lesions. In all of these teeth, the alveoli are still intact. Therefore, additional factors must be found to explain these findings.

The histologic picture of the teeth described in *figs. 1* to *9* indicates clearly that the teeth are still erupting. In such teeth the periodontal membrane is not yet fully adapted to withstand occlusal stress. Although the principal fibers of a tooth that has already reached the occlusal plane run chiefly in an oblique direction from the bone to the cementum, the corresponding fibers of a tooth that is still erupting are arranged more or less parallel to the root surface (6). In this stage, such a tooth is comparable to one whose periodontal membrane has been weakened by resorption of the alveolar wall, and therefore, at least temporarily, is unable to withstand masticatory stress. This accounts for the traumatic injuries in erupting teeth. In this connection, it is appropriate to recall the old observation of Tomes, quoted by Kronfeld (7), that resorptive changes in the deciduous teeth are not necessarily confined to the surface of the deciduous root that faces the permanent tooth germ, but may also occur on the opposite side on surfaces away from the growing permanent tooth. It may be that some of these resorptions are caused by traumatic injuries.

In the teeth of the older jaws, particularly in the jaw of $4\frac{1}{2}$ years of age, resorption of deciduous root and alveolar bone enters as an

additional factor in reducing the resistance of the periodontal support. This latter explanation has previously been offered by Bauer (4) and by Gottlieb and Orban (1).

Still later in the development of the dentition, the loss of deciduous teeth and the resulting additional occlusal load exerted upon the permanent teeth may cause injuries of the kind illustrated by Coolidge (2, figs. 4 and 7). In logical sequence this is then followed by the traumatic changes in the permanent dentition (8). Actual loosening or loss of teeth solely because of such traumatic injuries has as yet not been observed in either the deciduous or the permanent dentition.

The clinical and practical significance of traumatic injuries in the periodontal tissues of deciduous teeth must be considered from two angles. Early in life, at the time of eruption of the deciduous teeth and shortly thereafter, such traumatized teeth may perhaps be somewhat sore or slightly loose. This may lead to a temporary shift in mastication, which later on, in turn, allows the injured periodontal tissues to be repaired (*fig. 8*). As the traumatic damages are being repaired, there is a possibility of an ankylosis between deciduous root and alveolar bone. In this way the findings of Oppenheim (3), Kronfeld (7), and the observations of Noyes (9) in so-called submerging deciduous molars may be explained.

SUMMARY

Traumatic injuries found in the supporting tissues of 6 deciduous dentitions, ranging in age from 9 months to 4½ years, are reported in this study.

The least severe of these injuries consist of compression, ischemia, and hyalinization of the periodontal connective tissue. The most severe ones caused crushing and necrosis of the periodontal tissues and formation of hemorrhagic cysts. In most instances, the injuries were followed by undermining bone resorption and by evidence of tissue repair.

The etiology of these injuries is probably the weakness of the periodontal membrane of erupting and newly erupted teeth. During these periods, the peridontal tissues are not yet fully equipped to withstand masticatory stress. In the somewhat older jaws, the weakening of the periodontal support caused by shedding is an additional factor.

The clinical manifestations of such traumatic injuries in the deciduous dentition may be slight soreness of the erupting teeth, and, perhaps, later on ankylosis between teeth and bone by reparative formation of bone and cementum.

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SALIVA AND ENAMEL DECALCIFICATION

IV. Factors Involved in the Prevention of Caries

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In a preliminary report by the author (1) some observations were recorded of the acid neutralizing power of the whole saliva. In a second report (2) a method was described of collecting the mandibular (submaxillary and sublingual) saliva and the parotid saliva simultaneously in separate containers. The third report (3) showed the results of some experiments on the separated saliva on contrasting diets. Evidence of autolysis, fermentation and increase in the concentration of the H ions in the stagnant saliva was also reported. In this report the acid neutralizing power of the unstimulated separated saliva on the contrasting diets is shown as well as the protective action of the Ca and PO_4 ions in the saliva.

In order to study the acid neutralizing power of the saliva it is necessary to consider briefly the composition of the mineral phase of enamel and dentin. While the exact composition of the enamel and dentin is still a matter of controversy, considerable work has been done and the composition of these tissues has, at least approximately, been determined.

While the composition of whole teeth has been reported by a number of investigators, the paucity of information regarding the composition of enamel and of dentin has, no doubt, been due to the difficulty of separating these tissues. Among the more recent investigators in this field Crowell, Hodge and Line (4), who analyzed sections cut from extracted teeth including enamel, dentin and cementum, state that "All data from a variety of experimental studies, i.e., chemical, roentgenographical, crystallographical, etc., indicate that the inorganic parts of tooth tissues, whether enamel, dentine or cementum are one and the same substance." A single tentative empirical formula was proposed for the inorganic phase of enamel, dentin and cementum.

A method of readily separating enamel and dentin, described by Brekhus and Armstrong (5) enabled these investigators (6) to report the results of a sufficient number of analyses of unashed enamel and dentin to have statistical significance. Analyses of the enamel and dentin of a few teeth, from which the organic matter had been removed, showed that "The analytical composition of the protein free mineral phase of enamel is obviously different from that of dentine." In their table of ratios of the component parts of the mineral phase of enamel and dentin we find the following:

	Enamel	Dentin
$\frac{\%\ CA_3(PO_4)_2}{\%\ CaCO_3}$	12.60	7.41

These authors propose separate empirical formulae for enamel and dentin and state

"These formulas, in spite of their agreement with the analytical results, cannot represent the true state of combination of the elements in enamel and dentine. If the formula of the mineral phase of any calcified tissue is to be correctly represented, it is necessary to account for the fact that the x-ray spectograms of the mineral phases of all calcifications are practically identical with each other and with pure apatites (Taylor and Shepard, 1929). On the other hand, in view of the analytical differences between enamel and dentine and other calcifications, the data of x-ray analysis cannot be interpreted to indicate an exactly identical composition of the mineral phases of these materials.

"An explanation is needed of the fact that several substances may exhibit almost identical x-ray diffraction patterns and yet their composition be so diverse as to appear irreconcilable by the substitution of equivalent atoms in the apatite crystal lattice, as it is now conceived. Until such explanation is forthcoming, attempts to depict the composition of the inorganic fraction of bone, enamel and dentine as a single molecular species are futile. The results emphasize that the composition of the mineral deposited in calcified tissues is not absolutely fixed."

Recent reports from a number of investigators in this field indicate almost unanimous agreement that the principal inorganic component is hydroxyapatite.

A vast amount of research work has been done on the H-ion concentration of the freshly secreted saliva, but this factor is probably of only minor importance in determining the susceptibility or immunity to enamel decalcification. As so aptly expressed by Forbes (7)

"It should be noted that the neutralizing action of the saliva is not necessarily proportional to the H-ion concentration, i.e., pH of the saliva as is generally believed.—If the materials composing two buffer solutions are the same, and their pH is the same,

their neutralizing power will be proportional to the amount of buffer present. The neutralizing action of the saliva against acids depends upon not only the pH, but also the amount of buffers present."

The pH of the freshly secreted saliva is, however, a factor in determining the rapidity of the fermentation of the hydrolyzed carbohydrate. The lower the pH of the saliva, within certain limits, the more rapid the precipitation of the mucin, hydrolysis of the carbohydrate and proliferation of the acidogenic bacteria.

The time factor, in the opinion of the author, is one of the most important factors in this complicated process of enamel decalcification. Except where cleansing of the teeth is neglected, or there are inaccessible spaces around the teeth, or actual cavities exist which are not cleansed by brushing or mastication, the cycle of precipitation of mucin, hydrolysis and fermentation of the carbohydrate must take place within an average sleeping period of eight hours.

According to Forbes (8) "The main buffers of saliva are undoubtedly phosphates and carbonates." In addition to these the proteins probably have some buffer action.

In order to study the diurnal and nocturnal variations and the effect of the high and low carbohydrate diets described in a previous report (3) on the buffer action, or alkaline reserve, of the saliva, tests were made on the fresh, unstimulated parotid and mandibular saliva of the author, using the saliva separator (2), every 2 hours over a 24 hour period. The saliva was titrated with a 0.1 per cent lactic acid solution, using litmus solution as an indicator, as described in the preliminary report (1). *Fig. 1*, A and B shows the results of these tests. The vertical lines represent meal times; the abscissae represent the time at which the collection of saliva for that test was started and the ordinates represent the number of tenths of a milliliter of a 0.1 per cent solution of lactic acid necessary to produce a change of color in the saliva-litmus solution and was recorded as the alkaline reserve index. The light lines represent the parotid saliva and the heavy lines represent the mandibular saliva. Comparison of these charts shows a definite decrease in the alkaline reserve of the mandibular saliva on the high carbohydrate diet.

While these tests indicate the immediate neutralizing capacity of the saliva they do not show the principal factor in the protection of the

enamel against decalcification. The chief significance of a high alkaline reserve index is the delaying of the increase in H-ion concentration.

Clinically there are many types of enamel decalcification but micro-

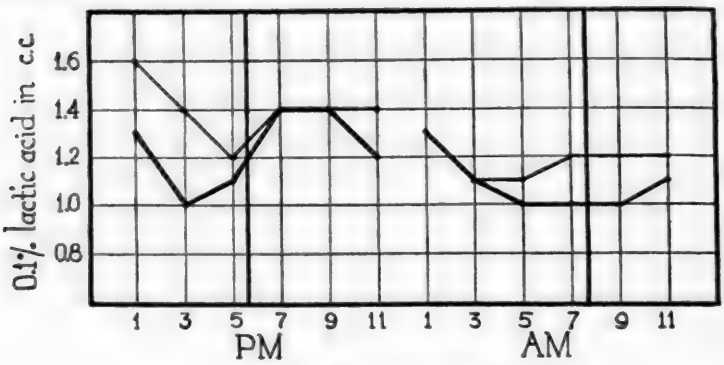

FIG. 1A. Low carbohydrate diet

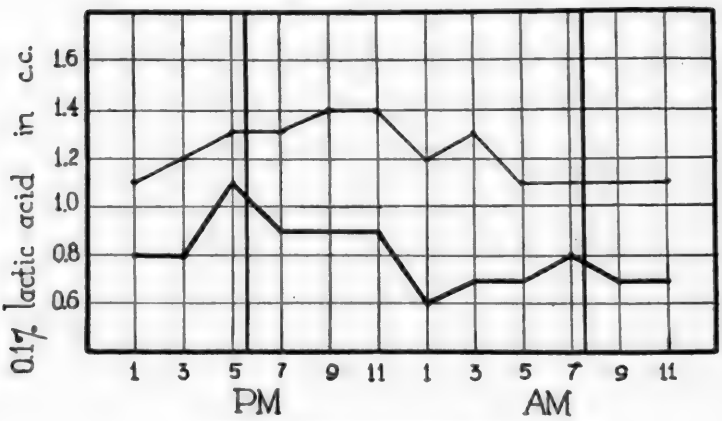

FIG. 1B. High carbohydrate diet

scopically there are only two, viz: 1. That in which the continuity of the enamel surface remains intact but a macroscopic white or opaque spot or area may be produced. Microscopically, decalcification of the intercolumnar substance of the enamel has taken place and, under favorable circumstances, penetrated through the enamel and dentine

to the pulp. This type is produced in the presence of an adequate concentration of H ions and an adequate concentration of calcium and phosphate ions in the saliva. 2. That in which decalcification starts on the surface of the enamel and progresses toward the amelo-dentinal junction with a complete destruction of the enamel prisms and interprismatic substance with the loss of surface continuity. This type is due to an adequate concentration of H ions and an inadequate concentration of calcium and phosphate ions in the saliva. (The term adequate is here used to express a sufficient amount to produce the condition under discussion.)

These two types of decalcification may alternate frequently or one type predominate, but almost every extracted tooth will show microscopic evidence of the 1st type on one or more surfaces of the enamel, usually more pronounced at the cervix on the labial or buccal surface.

It would seem that only one conclusion can be drawn from these two types of decalcification, which the author has produced *in vitro*, viz: in the 1st type only certain portions of the enamel, constituting a small percentage of the whole, are removed from the intercolumnar substance, allowing the enamel prisms to remain intact if undisturbed by external forces. Subsequent experiments will show that the $CaCO_3$ is removed allowing the calcium phosphate to remain. In the second type of decalcification the $CaCO_3$ and $Ca_3(PO_4)_2$ are removed simultaneously from the enamel prisms as well as the interprismatic substance.

To explain these phenomena we need only examine the work of Forbes (7) and of Enright, Friesell and Trescher (9). This latter group showed that the saliva is supersaturated in varying degrees with $Ca_3(PO_4)_2$ which is held in solution by the protein and CO_2. Quoting some of the conclusions of Enright, Friesell and Trescher, "The authors, then in the light of available information, accept 29.5— as the probable p.K.s.p. of tricalcium phosphate in saliva," p.K.s.p. being the negative logarithm of K.s.p. (solubility product constant). Again quoting the same group (9),

"There is not one case among 225 that had complete figures for calcium, phosphorus and H-ion concentration, in which the p.K.s.p. was higher than 28.81; the ion product, therefore, is always greater than that required for saturation. The average values for the

p-product of four different groups (arranged according to condition of teeth) are 25.91, 26.02, 26.13 and 26.26, starting with the group that had little or no caries and ending with the group that had considerable caries. All these results indicate not only a state of saturation but one of supersaturation. If this is true, if the saliva is supersaturated with tricalcium phosphate, why is there not a precipitation of the salt in excess of that required for saturation? All through their papers these workers show that the precipitation of tertiary calcium phosphate is a slow process."

There is much evidence, however, that tricalcium phosphate is sometimes precipitated from the saliva. Prinz (10) in his work on salivary calculus was able to get deposits of calculus in from 8 to 10 weeks on teeth outside the mouth by placing them in saliva, renewed daily, and kept at 40°C.

In his explanation of the manner in which decalcification of the enamel took place Forbes (7) states that

$$\underset{\text{Solid}}{Ca_3(PO_4)_2} \rightleftarrows \underset{\text{Solution}}{Ca_3(PO_4)_2} \rightleftarrows 3Ca^{++} + \underset{\text{ions}}{2PO_4^{---}}$$

which is a condition of dynamic equilibrium, and

$$(Ca^{++})^3 \times (PO_4^{---})^2 = K$$

in which K is an ionization constant.

Quoting Forbes (7),

"Since the enamel is primarily composed of inorganic salts we must assume that its solution follows the same lines as that of any other insoluble inorganic salt. Also, since the enamel is primarily composed of calcium and phosphates, we may assume for this discussion that it consists mainly of $Ca_3(PO_4)_2$, feeling that what holds true for this salt must hold true, with limitations, for the substance of which enamel is composed. We have seen that the solution of $Ca_3(PO_4)_2$ is dependent on the concentration of the Ca and PO_4 ions. If the product $(Ca^{++})^3 \times (PO_4^{---})^2$ is momentarily increased over the value K (ionization constant), deposition or precipitation of $Ca_3(PO_4)_2$ takes place; but if the product is reduced below K, solution of the $Ca_3(PO_4)_2$ proceeds until the product is again increased to K. It would therefore seem that those factors which influence the concentration of Ca and PO_4 ions, in the saliva bathing the teeth in any particular locality, must be determining factors in the etiology of caries.

"The chief factors involved in determining the PO_4-ion concentration of the solution bathing the teeth in any locality are likely to be (a) the phosphate concentration and (b) the H-ion concentration of the solution in that locality. The influence of the former is evident and requires no further comment, but a discussion of the latter may be in order. It was shown when considering the solution of $Ca_3(PO_4)_2$ in acids that the first step in the reaction was a reduction of the PO_4-ion concentration through a combination of the PO_4 ions with the H ions to form HPO_4 ions. This may be readily seen when one considers

the equilibrium equation representing the reaction $H^+ + PO_4^{---} \rightleftarrows HPO_4^{--}$. Since this is a state of dynamic equilibrium, it follows from the law of mass action that any increase in the concentration of either of the ions on the left hand side of the equation causes the equilibrium to shift to the right, with the formation of additional HPO_4 ions. For example, if the H-ion concentration is increased by the addition of acid, some of the H ions combine with the PO_4 ions, and a corresponding decrease in the concentration of the PO_4 ions ensues," and elsewhere in the same paper he states "Which by the further addition of acids may be changed into the soluble $Ca(H_2PO_4)_2$."

In a previous report (3) it was shown that when fermentation was prevented the concentration of fermentable sugar, or potential H ions, could be increased or decreased in the precipitated mucin of the author's saliva by increasing or decreasing the amount of carbohydrate in the diet. If fermentation had been allowed to take place in the presence of enamel, from the work of Forbes and of Enright, Friesell and Trescher, quoted above, we would have expected to find solution of the calcium phosphate of the enamel or precipitation of $Ca_3(PO_4)_2$ from the saliva, depending on the concentration of the H ions and the degree of supersaturation of the saliva with $Ca_3(PO_4)_2$.

EXPERIMENTAL

As it was impossible to obtain sufficient precipitated mucin for the following experiments, stimulated whole saliva was used in which the H ions were allowed to gradually accumulate by the fermentation of dextrose in the presence of an ample supply of potential Ca and PO_4 ions, available in the form of finely powdered $Ca_3(PO_4)_2$. About 100 extracted teeth were, at various times, placed in a mixture of 25 cc. of the author's stimulated saliva, 250 mgms. of dextrose and 1 gram of tricalcium phosphate (Merck's) and kept in the incubator at 37°C., renewing the saliva-dextrose-tricalcium phosphate (S-D-T) mixture when the sugar had been fermented as shown by the Benedict's qualitative test.

The teeth were selected on the basis of their macroscopic perfection of the enamel in the area selected to be exposed to the S-D-T solution. No history accompanied these teeth and most of them were encrusted with calculus. The teeth were thoroughly cleansed by scraping off all deposits and adhering tissue and polished with a stiff brush revolved on a laboratory lathe, using the finest powdered pumice. They were then placed in ether for several hours and finally in acetone, after which they were dried with warm air from a dental hot air syringe and coated with a 10 per cent solution of nitrocellulose (20 seconds viscosity), to which was added camphor, as a plasticiser, in the amount of 20 per cent of the weight of the nitrocellulose, the solution being colored dark red by adding 5 mgms. per cc. of duPont's oil red dye which is soluble in acetone. The teeth were coated with the nitrocellulose solution with the exception of a small strip of enamel about 1.5 mm. wide extending mesio-distally across the labial surface of the anterior teeth and the buccal and lingual surfaces of the posterior teeth.

Fifty extracted teeth, having a total exposed area of enamel of 100 to 150 sq. mm. were placed in the S-D-T solution and placed in the incubator at 37° C. While the changes

in pH of the solution varied slightly in numerous tests, fig. 2 represents the average changes which took place during a period of fermentation.

The teeth were placed in the S-D-T solution about 5 or 6 hours after placing the solution in the incubator, when fermentation was well established. After 48 to 72 hours of incubation the teeth were placed in a fresh solution, all of the dextrose having been fermented in the previous solution.

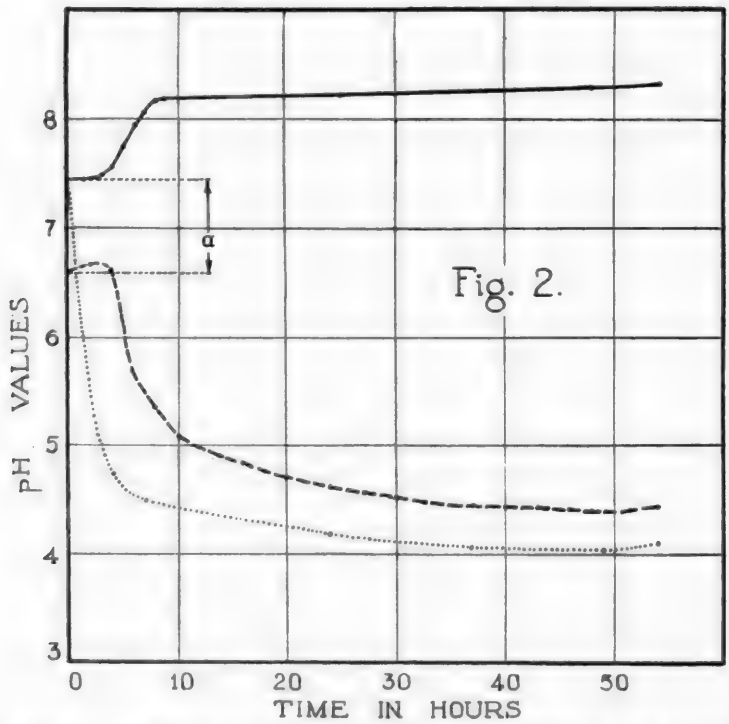

FIG. 2. ——, Changes in pH of the author's saliva. - - - - Changes in pH of 25 cc. of the author's saliva to which was added 250 mgm. of dextrose and 1 gm. of $Ca_3(PO_4)_2$. "a" represents decrease in pH upon agitating with tricalcium phosphate. ..... Changes in pH of 10 cc. of the author's saliva to which was added 100 mgm. of dextrose. All incubated at 37°C.

While the area of enamel surface exposed made very little difference in the changes in the pH of the S-D-T solution, the time required to ferment the dextrose was in inverse proportion to the area of enamel surface exposed.

Figs. 3, 4, 5 and 6 show photomicrographs of 4 lower incisors, of a number of specimens in the writer's possession, with exposed areas on the labial surfaces, which were subjected to the action of the S-D-T solution for different lengths of time, showing decalcification of the interprismatic substance without dissolution of the surface of the enamel. Some of the specimens show changes in the dentin.

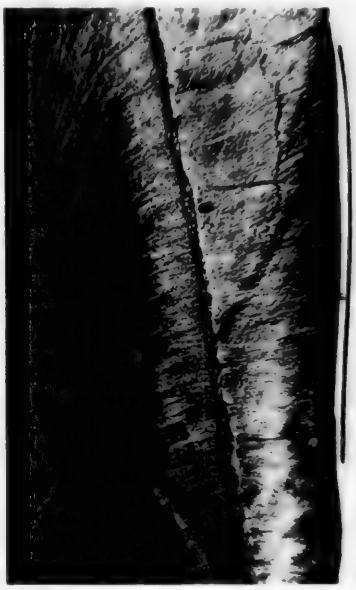

FIG. 3. Line indicates area on labial surface of mandibular incisor exposed to saliva-dextrose-tricalcium phosphate solution for 3 months. Shows type 1 decalcification and remineralization. Unstained.

Figs. 7, 8 and 9 show higher magnification of the decalcified areas of the teeth shown in *figs.* 4 and 5. All of the characteristics of incipient caries are here present, viz.: transverse striations, accentuation of the striae of Retzius and decalcification of the interprismatic substance without decalcification of the prisms, or, to be more exact, the enamel globules.

It seems evident from these photomicrographs that a part of the mineral phase of the intercolumnar substance of the enamel has been

removed without materially altering the enamel structure. While decalcifying the teeth in the S-D-T solution it was observed that considerable gas was formed as long as fermentation continued. To ascertain if this gas was caused by the action of the acids on the enamel, 50 cc. of stimulated whole saliva were collected and divided into two

FIG. 4

FIG. 5

FIG. 4. Line indicates area on labial surface of mandibular incisor exposed to saliva-dextrose-tricalcium phosphate solution for 5 months and 10 days. Shows type 1 decalcification and remineralization of enamel and changes in the structure of the dentin. Unstained. Higher magnifications of the circular areas are shown in *figs.* 7 *and* 9.

FIG. 5. Shows same as *fig.* 4 exposed to the S-D-T solution for 10 months. Unstained. Higher mag. of circular area is shown in *fig.* 8.

equal portions, (A) and (B), and 250 mgms. of dextrose and 1 gram of tricalcium phosphate added to each and both placed in the incubator for 30 hours to expel the suspended and combined CO_2 in the saliva. At the end of 30 hours a tooth, with only macroscopically sound enamel exposed, which had been placed in 10 per cent lactic acid for 10 minutes to decalcify the outer resistant layer of enamel, was placed in A and

nothing added to B, which was used as a control. From each flask a glass tube, with an outside measurement of about 4 mm., extended upward through a rubber stopper and terminated in a U shape. A small hole in each stopper permitted the passage of air while adjusting the apparatus. The U shaped portions of the glass tubes were filled

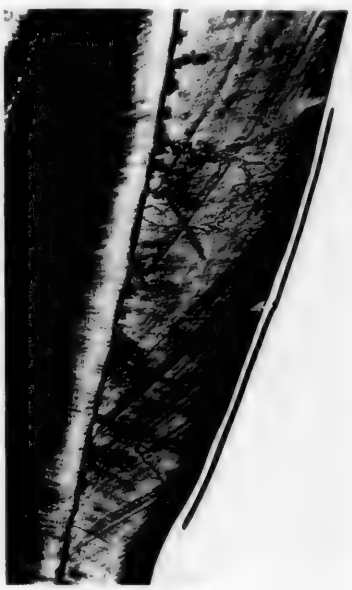

FIG. 6

FIG. 7

FIG. 6. Shows type 1 decalcification and remineralization. Exposed to S-D-T solution for 1 year. Unstained.

FIG. 7. Higher magnification of area marked 7 in *fig.* 4. Shows decalcification of intercolumnar substance.

with water and the holes in the stoppers sealed. Both A and B were placed in the incubator at 37°C. After 4 or 5 hours the water in the U-shaped tube of A, which contained the S-D-T solution and the tooth with only enamel exposed, was entirely displaced, while in B, containing S-D-T solution only, the water in the U tube had remained stationary.

Having determined that a gas was formed by enamel in the incubated saliva containing dextrose and tricalcium phosphate, a similar apparatus was constructed except that the gas was passed through a saturated solution of $Ca(OH)_2$ before entering the terminal U-shaped tube containing water, which was enlarged at the end to prevent the

FIG. 8

FIG. 9

FIG. 8. Higher magnification of area marked 8 in *fig.* 5. Shows decalcification of intercolumnar substance.

FIG. 9. Higher magnification of area marked 9 on *fig.* 4. Shows decalcification and remineralization.

water from escaping and prevent the $Ca(OH)_2$ from coming in contact with the air. After incubating for 19 hours examination of the beaker containing the $Ca(OH)_2$ showed a white precipitate at and below the mouth of the tube leading from the flask containing the S-D-T solution and the tooth with only enamel exposed. As in the previous experiment the S-D-T solution had been incubated for 30 hours before the tooth was added.

From the above experiments it would appear that the carbonates ($CaCO_3$) are dissolved out of the intercolumnar substance of the enamel and apparently sometimes from the dentin in the presence of an adequate concentration of H, Ca and PO_4 ions in the saliva without dissolving out the $Ca_3(PO_4)_2$ of the enamel.

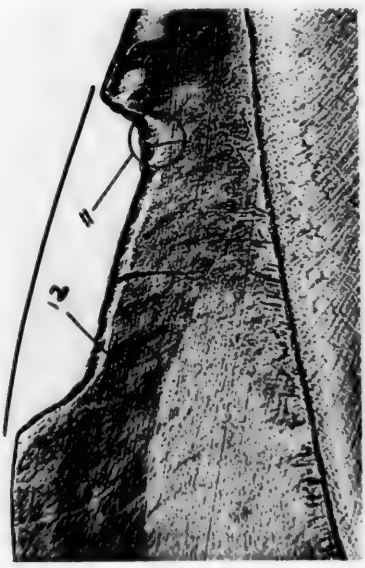

FIG. 10

FIG. 11

FIG. 10. Type 2 decalcification. Molar tooth in saliva containing dextrose, but no $Ca_3(PO_4)_2$ for 3 weeks.

FIG. 11. Higher magnification of area marked 11 in *fig.* 10.

From the photomicrographs shown in *figs.* 4, 5 and 6, and others in the author's possession, it is evident that the amount of decalcification of the enamel and dentin bears no relation to the length of time that these tissues were subjected to the action of the S-D-T solution. The chemical composition of the mineral phase of enamel and dentin is, therefore, probably the determining factor in the amount of decalcification which takes place in the presence of an adequate concentration of H, Ca and PO_4 ions in the saliva and we may conclude that the

striae of Retzius are hypoplastic areas in which the $Ca_3(PO_4)_2/CaCO_3$ ratio has been decreased, probably due to some disturbance of normal mineral metabolism.

Having considered the effect of the accumulation of H ions on the enamel when the saliva remains saturated with Ca and PO_4 ions, or

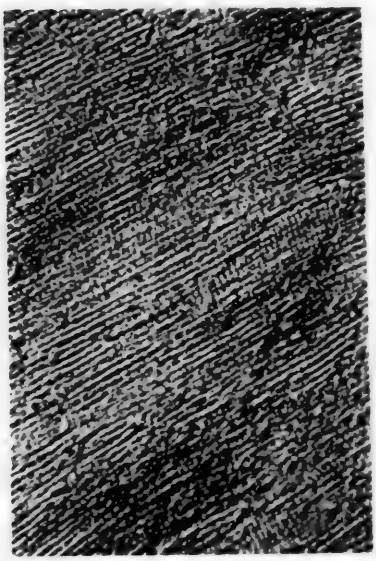

FIG. 12

FIG. 13

FIG. 12. Higher magnification of area marked 12 on *fig.* 10.

FIG. 13. Shows tooth exposed to Saliva-Dextrose sol. for 2 weeks. pH was not allowed to go below 4.50. Compare with *fig.* 6 in which the tooth was exposed to S-D-T for 1 year, 4 months of which the pH was below 4.50.

the first type of enamel decalcification, let us now consider the second type in which there is an accumulation of H ions but there is an inadequate supply of Ca and PO_4 ions. *Fig.* 2 shows the changes in the pH of the author's saliva under these conditions during a period of fermentation. No $Ca_3(PO_4)_2$ was added to the saliva and dextrose. It will be observed that there was a rapid decrease in the pH during the first 4 hours, after which there was a gradual decrease to pH 4.07.

In this test 10 cc. of saliva and 100 mgms. of dextrose were used and approximately 6 sq. mm. of enamel were exposed to the solution.

Fig. 10 is a photomicrograph of an exposed area of a molar tooth which was kept in the above solution for 3 weeks, the solution being renewed when all of the sugar was fermented. Although the sugar concentration was the same as in the 1st type of enamel decalcification, it is evident from this, as well as other specimens in the author's possession, that the enamel rods as well as the intercolumnar substance were dissolved away, but dissolution of the intercolumnar substance apparently preceded that of the enamel prisms.

Fig. 11 is a higher magnification of *fig.* 10 showing the disintegration of the enamel at the exposed surface. *Fig.* 12 is also a higher magnification of *fig.* 10 showing decalcification of the intercolumnar substance about half way between the disintegrated surface and the amelo-dentinal junction.

DISCUSSION

If we compare type 1 decalcification (*figs.* 4, 7 and 9) with type 2 (*figs.* 10, 11 and 12) we find that, although decalcification has occurred in both types, they present different appearances under the microscope. In the 1st type the decalcified area has become darker, especially in the Striae of Retzius, while in the 2nd type close examination shows that the decalcified area has become more transparent. If the darker appearance of type 1 is due, as is believed, to the organic matter, why does it not present the same appearance in the 2nd type?

Referring again to our ionic equation $Ca_3(PO_4)_2 \rightleftarrows 3Ca^{++} + 2PO_4^{---}$ Osborne, Noriskin and Staz (11), state

> "In neutral media the proportion of ions is infinitesimal, but as the medium is made more acid, the change from left to right is speeded up, with the result that more salt dissolves. By the mass-action law, the rate of change from ions to solid is proportional to the product (concentration Ca^{++})^3 × (concentration PO_4^{---})^2. For a given strength of acid, i.e., for a given pH the change from left to right will be constant; but the change right to left will be fast if the acid contains many calcium and phosphate ions and slow, if it contains few or none."

We will assume that the saliva is supersaturated with $Ca_3(PO_4)_2$ and the precipitated mucin in a localized area contains potential H ions in the form of a complex carbohydrate; hydrolysis and fermenta-

tion of the carbohydrate take place with an increase in the concentration of the H ions. With the increase in the H-ion concentration dissolution of the $CaCO_3$ takes place, resulting in an increase in the concentration of the Ca ions with an increase in the ion product over the value K, and a decrease in the H-ion concentration with a shift in our ionic equation from right to left. As a result of these changes in Ca and H-ion concentrations $Ca_3(PO_4)_2$ is deposited at the point of dissolution of the $CaCO_3$, producing remineralization. This is in accord with the conclusions of Andresen (12) in his studies of decalcification and recalcification by means of polarized light.

Remineralization may be partial or complete. If remineralization is incomplete the enamel is rendered more or less porous and penetrable to the oral fluids and becomes less resistant to mechanical abrasion and susceptibility to further decalcification is increased.

If the potential Ca and PO_4 ions in the saliva are inadequate, due to an excess of potential H ions, there is produced a condition in which the ion product is below the value K and $Ca_3(PO_4)_2$ as well as $CaCO_3$ is dissolved, producing the 2nd type of enamel decalcification.

That the concentration of the Ca and PO_4 ions in the saliva is the determining factor as to the type of decalcification is indicated by comparing *figs.* 6 and 13. The tooth shown in *fig.* 6 was subjected to the action of the S-D-T solution for 1 year; reference to *fig.* 2 will show that the pH of the solution was below 4.50 for 40% of the time, or a conservative estimate of 4 months. *Fig.* 13 shows a lower incisor that was subjected to the action of saliva and dextrose for 2 weeks, but the pH was not allowed to go below 4.50 at any time. Each day the tooth was placed in a fresh saliva-dextrose solution which had been incubated until the pH reached 5.50 and allowed to remain until the pH reached 4.50 and a record kept of the number of hours.

The two types of enamel decalcification herein described may alternate frequently, or one type may predominate at one period (hour, day, month, year or period of life) and the other type, or no decalcification, at another period.

The confusion existing in dental literature regarding the interpretation of the work of investigators, using different methods (microscopic, Grenz-ray, polarized light, chemical analysis, etc.), may be

the result of changes in salivary composition producing mixed types of decalcification. With the ability to produce relatively pure types of these two forms of decalcification more satisfactory results should be obtained in the future.

The saliva of the author was used exclusively throughout these experiments and its composition altered in conformity with predetermined variations that were produced by diet and other factors. All pH determinations were made with the glass electrode on the Beckman pH meter.

The author wishes to acknowledge the assistance of his son, Robert T. Gore, B. S. in making many of the tests described in this and the preceeding papers in this series, and his advice on numerous occasions.

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DECOMPOSITION OF CARBOHYDRATE SUBSTRATES BY DENTAL PLAQUE MATERIAL

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We believe, as do many investigators, that the pathogenesis of dental caries may be explained best by the assumption that dental plaque material, under certain conditions, initiates the early lesions in enamel. Plaque material is a thin film which adheres tenaciously to the tooth surface and cannot be dislodged by a stream of water or by ordinary swabbing or brushing. It can be removed by scraping with a scaler, and upon gross examination exhibits a white amorphous appearance. Microscopically, plaque substance is seen to be composed of a mass of bacteria embedded in a matrix of filamentous microorganisms and organic debris; the bacterial bodies appear to make up perhaps half of the total bulk of the plaque. There is a characteristic flora associated with plaques covering areas of initial caries.[1]

Despite the importance of plaque material in the caries problem few investigations, other than morphological, have been conducted upon it. An investigation is presented here of the ability of plaque material to decompose the more important carbohydrate substrates. We have studied plaque material removed from teeth of caries-active and caries-free individuals. However, no attempt was made in any one individual to obtain plaque substance separately from the so-called susceptible or immune areas which are recognized clinically. That is, in these experiments we have studied composite samples of plaque material from the same individual, so that the substance obtained from caries-active individuals includes plaques from areas undergoing active caries, as well as from others which were entirely free from the disease. In a later study, we intend to give compara-

[1] Blayney, J. R., Kesel, R. G., and Wach, E. C., *J. D. Res.* 15: 326, 1936.

tive data on the metabolism of plaques from "immune," "susceptible" and "active" areas in the same individual.

We do not assume that the acid produced by plaque material necessarily causes the lesions in dental caries. No uncontroversial evidence is available for this assumption. Therefore, *pro tem*, the production of acid by the plaque material should be viewed as an index of activity of intra- and extracellular enzyme systems in the plaque, rather than as the mechanism of the chemopathogenesis of dental caries.

EXPERIMENTAL

The tooth surfaces were cleaned of all superficial debris by gentle rubbing with cotton swabs and by washing with a stream of water. The teeth were isolated with cotton rolls and dried with a current of air. The plaque material was then scraped off with a scaler, immediately placed in 0.45 cc. of distilled water, and suspended uniformly by means of a small, motor-driven homogenizer. Sufficient plaque substance was used to give a suspension containing 1 to 3 mg. dry weight of material in 0.45 cc. Of this suspension, a 0.2 cc. portion was employed for determination of initial pH and lactic acid content; to the remainder, solid glucose was added to give a concentration of 0.25 per cent. The mixture containing the added glucose was incubated at 37°C. for 30 minutes, cooled to room temperature, and tested for pH and lactic acid content.

A micro glass electrode was used for making pH determinations. Lactic acid (total lactate) was determined by the method of Miller and Muntz[2] with modifications as follows: 0.2 cc. of the plaque suspension is pipetted into a centrifuge tube containing 0.027 cc. of $CuSO_4$ solution (saturated $CuSO_4$ diluted with an equal volume of water). To this mixture 6.6 mg. of solid $Ca(OH)_2$ are added, stirred thoroughly, and allowed to stand for 20 minutes with occasional agitation. After centrifugation at 3,000 r.p.m. for 10 minutes 0.1 cc. of the supernatant fluid is removed and analyzed according to the procedure described by Miller and Muntz. The lactate values in the tables are recorded in terms of the total original suspension (e.g. 0.45 cc.). Experiments in which small quantities of lactate were added to plaque material demonstrated a recovery of 95 per cent by this modified method.

RESULTS

Lactic and Total Acid Production in Glucose Solutions. The lactic acid production and pH decrease, upon incubation of the plaques in glucose solution, are given in Tables I and II for composite samples obtained from "caries-free" and "caries-active" individuals. Caries-free individuals were classified as such when they showed negative findings upon clinical and radiographic examinations, and when, in addition, they gave total saliva counts[3] for lactobacilli and yeasts of

[2] Miller, B. F. and Muntz, J. A., *J. Biol. Chem.* **126**: 413, 1938.
[3] Hadley, F., *J. D. Res.* **13**: 415, 1933.

less than 1000 per cc.; the caries-active cases were consistently positive by these three criteria.

The results in Tables I and II show that plaque material obtained from both types of individuals acts upon glucose to produce lactic acid. The capacity to decompose glucose to lactic acid appears to be the same in both groups. The production of acid varied with the

TABLE I

Acid production on glucose substrate, caries-free individuals

LACTIC ACID PRODUCTION (RANGE)	PATIENT NO.	LACTIC ACID, INITIAL	LACTIC ACID, FINAL	LACTIC ACID, INCREASE	pH INITIAL	pH FINAL	pH DECREASE
		γ	γ	γ			
0–10 γ	9	1.0	7.5	6.5	7.32	6.20	1.12
	10	0.5	3.2	2.7	7.00	6.20	0.80
	13	2.8	5.4	2.6	6.53	5.75	0.78
	15	0	8.0	8.0	6.65	5.14	1.51
	16	0	5.4	5.4	6.82	5.20	1.60
	39	2.8	8.0	5.2	7.18	6.00	1.18
	46	0.9	2.8	1.9	6.69	6.04	0.65
	50	2.8	7.4	4.6	7.18	6.48	0.70
10.1–25 γ	5	0.8	15.9	15.1	6.60	5.06	1.54
	8	1.5	14.5	13.0	7.60	6.10	1.50
	11	1.5	11.8	10.3	6.63	4.50	2.10
	12	1.0	12.3	11.3	6.48	4.17	2.30
	14	1.5	19.8	18.3	6.55	4.65	1.90
	23	1.0	11.5	10.5	5.80	4.78	1.02
	17	0.8	22.0	21.2	6.40	4.22	2.18
	54	1.4	18.7	17.3	6.47	5.11	1.36
	69	9.9	22.6	12.7	6.70	5.95	0.75
25.1—over γ	43	3.0	56.8	53.8	6.86	5.26	1.6
	48	1.7	32.4	30.7	6.79	4.59	2.2
	73	13.3	46.1	32.8	6.92	5.34	1.58

quantity of plaque substance. Only a few caries-free individuals with large amounts of plaque material were available for study. When such subjects were found, e.g. numbers 43, 48 and 73 in Table I, it was observed that their plaque material produced large quantities of acid. All of the 19 individuals who satisfied the criteria for the absence of active caries possessed plaque material which produced significant quantities of lactic acid and caused a definite drop in pH (Table I).

The drop in pH after incubation of plaque substance in glucose follows the same pattern as the lactic acid production, and does not

TABLE II

Acid production on glucose substrate, caries-active individuals

LACTIC ACID PRODUCTION (RANGE)	PATIENT NO.	LACTIC ACID, INITIAL	LACTIC ACID, FINAL	LACTIC ACID, INCREASE	pH INITIAL	pH FINAL	pH DECREASE
		γ	γ	γ			
0–10 γ	18	0.6	7.2	6.6	6.50	5.30	1.20
	22	4.3	9.6	5.3	6.80	5.40	1.40
	20	0	6.0	6.0	7.10	5.70	1.40
	21	0	8.0	8.0	7.20	5.80	1.40
	25	1.0	7.7	6.7	6.48	6.07	0.41
	26	0.5	7.2	6.7	6.86	5.35	1.51
	41	2.8	10.5	7.7	7.09	5.58	1.51
	53	2.8	5.7	2.9	6.75	6.20	0.55
	70	21.4	25.6	4.2	6.41	5.70	0.70
	59	2.8	9.9	7.1	7.10	6.87	0.23
10.1–25 γ	6	1.9	20.0	18.1	6.42	5.26	1.16
	24	2.3	24.6	22.3	6.60	4.49	2.10
	22(a)	2.9	17.1	14.2	6.65	5.58	1.07
	32	2.0	14.8	12.8	7.01	5.36	1.65
	40	1.1	16.4	15.3	7.05	5.71	1.34
	44	10.2	22.7	12.5	6.83	4.85	1.98
	45	10.7	25.3	14.6	6.55	5.28	1.27
	51	2.8	15.6	12.8	6.48	5.03	1.45
	52	2.3	22.7	20.4	7.20	5.92	1.28
	74	17.3	38.9	21.6	7.20	6.36	0.84
	71	3.6	14.5	10.9	7.18	6.40	0.78
	72	6.7	29.0	22.3	6.80	5.80	1.00
25.1—over γ	4	1.9	28.1	26.2	6.38	6.00	0.38
	7	2.2	40.1	39.9	6.42	4.78	1.64
	47	2.3	29.0	26.7	7.19	6.51	0.68
	49	3.3	35.8	32.5	6.90	4.99	1.91
	65	31.1	101.8	70.7	6.40	4.96	1.44
	61	9.2	56.4	45.2	6.70	5.11	1.59
	75	5.5	47.3	41.8	7.12	5.52	1.60
	58	17.0	96.4	79.4	6.79	5.74	1.05
	67	8.6	38.1	29.5	6.78	5.10	1.68
	68	15.9	50.8	34.9	6.10	4.91	1.19

reveal any difference between the composite samples obtained from caries-free and caries-susceptible individuals. There is no quantitative relationship between lactic acid production and pH decrease.

This may be due to differences in buffer capacity of the plaque substance, or the production of acids in addition to lactic.

Activity of Plaque Material on Disaccharides and Starch. Because the teeth are exposed to carbohydrates such as sucrose, maltose, lactose and starch in the diet as well as glucose, it was of interest to determine whether or not plaque material produces acid from these compounds, and if so, to compare the rate of acid production with that obtained on the simpler substrates, glucose and fructose. Direct comparison of the activity on the various substrates was made by

TABLE III

Lactic acid production on various carbohydrate substrates

CASE NO.	GLUCOSE	FRUCTOSE	SUCROSE	MALTOSE	LACTOSE	STARCH
58	79.4		96.5	76.6	32.0	
61	45.2		45.2	51.3	15.9	39.5
65	70.7		92.9	67.7	47.9	33.9
69*	12.7	14.3	19.9	13.9	0.8	3.9
76		68.1	70.3	57.7	19.8	51.7
81*		25.5	48.1	23.6	14.6	7.3
82		106.5	132.0	101.7	41.3	73.1
83		130.9	165.0	93.6	10.2	74.4
85	124.5	116.8	138.0	107.1	55.1	56.8
86	285.6	227.6	312.6	225.6	86.8	182.1
87*	94.6	104.2	127.4	85.0	23.2	81.9
88*	106.1	102.2	131.2	81.0	36.6	51.9

The quantities of lactic acid are given in micrograms for the entire initial suspension of plaque substance in 1.5 cc.

* Caries-free individuals.

removing a large quantity of plaque material from many teeth of one individual, and homogenizing the entire quantity in 1.5 cc. of water. This volume was large enough to permit determinations on 5 substrates. To a 0.2 cc. aliquot of suspension, each of the disaccharides was added in solid form. Starch, freed from reducing sugar by dialysis, was added as a 5.5 per cent solution. In each case the final substrate concentration was 0.5 per cent. The quantities of lactic acid produced during a 30 minute period of incubation on the various carbohydrate substrates are shown in Table III. These results demonstrate that plaque material produces lactic acid with each substrate, especially well with sucrose and maltose, and even

very rapidly with such a complex carbohydrate as starch. It is shown by these results that disaccharides such as sucrose and maltose can be decomposed as readily by the plaque material as glucose and fructose. Possibly the limiting factor for these 3 compounds is the rate at which the monosaccharides can be phosphorylated.

The experiments summarized in Table III were performed for the most part on caries-active patients because as a rule only such patients provide sufficiently large quantities of the plaque material. However, enough cases are included in the table to indicate that plaque substance from caries-free individuals decomposes the disaccharides and starch in the same manner as the material from active cases.

Decreases in pH after incubation on all these substrates were observed, and they were of the same order of magnitude as those listed in Tables I and II. No significant differences in pH drop were elicited between the caries-active and caries-free individuals on any of the various carbohydrate substrates.

CONCLUSIONS

Composite samples of plaque material obtained from teeth of caries-free and caries-susceptible individuals produce lactic acid when incubated in glucose solutions. The quantity of lactic acid and the total pH drop vary with the amount of plaque material. Caries-free individuals possess plaque substance which produces acid *in vitro* as readily as composite samples from caries-active subjects. Also, plaque material from both types of individuals produces lactic acid from sucrose and maltose as readily as from glucose, and to a lesser, but still very definite extent, from lactose and starch substrates.

THE ESSENTIALS OF CARIES STATISTICS AND A SUGGESTION FOR A STANDARD

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It is a matter of general knowledge that researches into the statistics of caries have not produced the results we might wish for and that the figures obtained so far cannot be interpreted in an unequivocal sense. Presumably, we get higher values if we include incipient caries than we do when we set the limit at a more or less striking stage. To a greater or smaller extent, percentage differences between data can therefore be attributed to differences in the delimitation of the condition itself. However, some investigations into the frequency of dental caries have yielded information of interest. This is specially true of inquiries based on more aberrant sources, as for instance from Tristan da Cunha, Eskimos, etc., but, when we turn to comparisons, statistical research on caries statistics in civilized countries has not been able to surmount the methodological difficulties. As a result, the majority of problems dealing with frequency of caries are unsolved. Decisive conclusions cannot be drawn from individual cases with reference to the significance of heredity and environment. A solution must be sought by the statistical approach. We need information on the frequency of caries in pregnancy, in different parts of a country when people live under different conditions, in different social classes, and, above all, on the rapidity with which it progresses in teeth in different circumstances among people of both sexes and different ages. These are mentioned merely as an example of the significance which dependable statistical survey of caries may eventually assume.

Our problem is to get away from a subjective viewpoint towards dental caries and to try to get as objective a measure as possible. If caries does not belong to the class of characteristics which can be measured on an ordinary scale, as we can measure the stature of an individual, it may also be pointed out that we can never get an exact

value for the latter. When we do measure stature, we really make a classification and state that an individual's height falls within a particular range of our yardstick. In other words, his or her score lies inside particular limits, and if it extends below or above these limits it is necessary to assign a different figure. Thus, it may be asked whether it is possible to use, for dental caries, a similar method with reasonable claims to precision.

It would be satisfactory to be able to state approximately the volume of crown destroyed in proportion to the total volume, but it is obvious that this is not possible because the original position of the surface cannot always be decided. In some cases it can be reconstructed with confidence, but in many it is quite impossible to do so. In such circumstances all that remains is to try to assign an estimate by some more rough and ready method. In any case an attempt must be made to settle limits corresponding to degrees on a scale and, so far as possible, independent of subjective bias. In principle the problem is of the same sort as defining eye or hair color, as well as for statistics of some morbid changes such as goiter. When it is necessary to deal with characteristics of this kind it is not possible to arrive at a measure which depends on a particular scale, more or less directly, related to the metric system. So far as eye color is concerned, it has been possible to devise serviceable methods by the use of glass eyes of different colors. Eye colors are then classified without great difficulty by deciding to which model the test subject is closest. In a similar way the difficulties of measuring hair color have been resolved. The hair of an individual is compared with a scale of test hairs of different shades, arranged in an appropriate way. It is then easy to assign the limits between which the color of a particular person's hair lies.

Such scales for hair and eye color have been used in research work on the inheritance of bodily characteristics. Of special interest from this point of view is work by Hogben and his collaborators on color change in cold blooded animals. This furnishes a striking example of how the use of such an arbitrary standard brings inquiries which have been circumscribed by personal impressions within the scope of quantitative experimental treatment. For the Hogben melanophore-index, arbitrary numbers are assigned to different configurations of the pigment cells, as seen in microscopic examination. So subjective

assertions about the visible shade of an animal are superseded by ratings for which the personal error is small and can be assessed. It is thus possible to construct time-graphs of normal responses or tolerance to endocrine extracts, and in this way the rôle of nervous impulses or hormone secretion in the control of color change has been explored with results which could never have been obtained without recourse to an objective measure of the phenomenon itself.

The suggestion here made is that such a procedure should be utilized for caries. What may be called a caries standard should be established and with the aid of this standard caries can be classified. It would be based on models, i.e. artificial teeth of natural size, imitating decay of varying extent. Then it would be possible to state that a decayed area of a particular tooth corresponds with a particular model or that the extent of it lies between two of them. Both methods of scoring are serviceable, but in this connection it may be emphasized that it is better, where possible, to state that decay in a particular case lies between two limits than to score it according to the model with which it agrees most closely. There is no need to go into the details of such a scale except to emphasize that when the model which agrees most closely with the condition observed is specified it can be taken for granted that the tooth diverges from it more or less, and it is not known precisely how wide the divergence may be, i.e. how coarse the class is. But if it is said that the decay of a tooth lies between the limits defined by two models, the boundaries are clear, and it is therefore being stated that a particular tooth shows more decay than a particular model and less than, or at most as much as, another one.

When it comes to the practical task of constructing a caries standard, it is essential to choose appropriate boundaries. This ought to be done in such a way that an appropriate number fall within the limits so defined. If the limits lie too far apart, too many cases will lie between them. This is unsuitable from the standpoint of statistics. If the limits lie too close together, too few cases will lie within each class and this means that it will become necessary to lump classes together when the material has been worked up, compelling unnecessary work. If data are distributed on a normal curve of error, the limits should be chosen so that each class embraces $\frac{1}{3}$ of the standard

variation, i.e., $\frac{1}{6}$ of the entire range. In reality there is reason to suspect that caries would not be distributed on a probability curve, if a classification were used which seems plausible on general grounds. This means that the problem of appropriate class magnitude is a little more complicated than one is disposed to assume at first sight.

It is just because it is necessary to take account of statistical criteria, that a scale must be constructed in conformity with the behavior of the material it is intended to investigate. The way to set about this is to start by making models which seem to be suitable. After this the distribution of a sufficiently large sample in different classes can be tested. According to the result obtained, the limits must then be more or less rectified. To some extent therapeutic criteria may be considered in delimiting the grades, and it is also necessary to have separate sets for deciduous and permanent teeth. From what has been said, it follows that it is by no means a trivial task to arrive at a serviceable standard suitable both for dental and statistical research.

It is clear that the behavior of different material or of similar materials in different lands can vary, but the divergences in civilized countries should not be so great as to prevent the use of a single standard. This could carry with it great advantages, and the aim should be directed at this even if detectable differences show up. The difficulties of reaching international agreement are always great, but, even if there is delay before such a scale is accepted, the logical grounds which support such agreement seem to be so decisive that it is not necessary to doubt the possibility of reaching unanimity in the long run. With regard to the practical use of a caries standard, it should be emphasized that those who are going to undertake field work should start off with organized instruction, and this should begin with exercise in classifying a collection of models. Afterwards they could proceed to deal with living materials, and every research worker should finally carry out a parallel investigation with another one involving the simultaneous classification of a hundred individuals. By such means figures can be obtained for the way in which individual judgments differ, that is to say, a measure of the systematic errors. Such differences can be used for correction factors to adjust figures obtained in an inquiry carried out by several persons in accordance with results which would be obtained if the same investigation had been made by a

single person. It might well happen that, when good field workers are available, personal bias would not to any great extent lead to divergences large enough to necessitate recourse to such correction factors.

It must be obvious that the advantage which a caries standard conveys would be significant, and that it would furnish opportunity for more thorough and more reliable statistical analysis. With regard to scoring, it must be emphasized that it can best be done with the aid of figures. The suitability of using diagrams, different sorts of symbols and so forth has been discussed, but it is a fact that all statistical work must be based on information which is numerical in form, and that when numbers can be used they are the most suitable sort of symbols. In special circumstances other signs may have advantages, but with regard to a caries standard devised in the way suggested, numerical signs are, so far as the author can see, the most suitable one. It may be appropriate in practical work to reject some grades of the standard devised. For instance, one may only want a rough and rapid classification, and if so may reject some of the type limits of the original scale. Those which can be dispensed with, must also be decided with reference to the material investigated, and it is obvious that one should not reject any, if the time gained by doing so is insignificant.

Without an empirical orientation towards the way in which a caries standard works out in practice it is difficult to say anything more definite about this question. In the working out of the scale it must be remembered that caries can begin at different sites and can be of different types. Some preliminary models made for such a scale by Dr. Lindström (*fig.* 1) illustrate this. Clearly many problems of detail remain to be solved. The caries standard suggested by the writer implies the possibility of getting a satisfactory method of scoring teeth which have not been treated. Teeth which have been subject to treatment and have subsequently developed secondary caries raise a special problem, and extracted teeth entail a special difficulty which will not be discussed in this context. The time available to the writer has not made it possible to work out a more definite plan and to analyze all the details encountered.

It is not necessary to enter on a discussion of the statistical problems which working up caries data entails. In this field the problems are somewhat intricate. The literature which deals with it is treated in a

comparatively clumsy way and gives scant clues. When one reflects upon how much expense has been applied to caries statistics with

FIG. 1. Illustration of limits for a standard scale, showing central decay

scarcely any practical result, it seems to the author that it is well worth while to submit to the effort and cost required for setting up a serviceable scale for such work. From a scientific standpoint it is a

task which will pay in the long run; but it is naturally tempting for a scientific worker to try to arrive at a more facile solution. He wants to see results and can only devote a moderate amount of time and money to an investigation. From this point of view it is easy to understand that many have fallen into the temptation of compiling statistics about caries in spite of the fact that prospects of reaching conclusions of any great significance are not considerable, but from the viewpoint of science it is scarcely rational that cost and effort should be devoted to inquiries of which the majority must be defective. It is better to lay out once and for all a sum which would guarantee more dependable results at less total expense.

In medicine, as in dentistry, statistics have not enjoyed a high prestige and conversely statistical experts have not regarded statistics collected by physicians and dentists with greater respect. However it may now be hoped that conditions will change and if appearances do not deceive, we are already on the way to a better state of affairs. A prerequisite for the possibility of scientific progress in dental and medical statistics is to pay attention to statistical principles in the collection of the raw data. Too often we turn to the statistician only after the material has been collected, and the mistakes already made from a statistical point of view cannot be rectified. The object of this contribution is to set forth some of the principles essential to a more satisfactory basis for caries statistics from this point of view.

DENSITY AND REFRACTIVE INDEX STUDIES OF DENTAL HARD TISSUES

III. Density Distribution of Deciduous Enamel and Dentin[1]

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INTRODUCTION

There is no published material on density distribution of dry deciduous enamel and dentin. No density values on moist deciduous enamel are available, however Deakins, Boyd and Drain (1) have shown a density distribution for 51 moist blocks of deciduous dentin. They found an approximately normal distribution around the mean density of 1.91. Their values were on samples which included water and protein in the tubules and some cementum. Black (2) gave a value of 2.08 for moist dentin from one deciduous root.

The recent development of the method for separation of tooth tissues (3) into samples of known density limits provides an opportunity for measurement of these fundamental properties of deciduous enamel and dentin.

In the work reported herewith all of the enamel and all of the dentin from several teeth were pooled, dried, powdered and separated into fractions of known density limits. Density distribution curves were obtained and mean densities calculated for the dry tissues studied.

METHOD

The tooth samples were pooled teeth obtained from the extraction clinic of the Rochester Dental Dispensary, through the courtesy of Dr. H. J. Burkhart and Dr. E. J. Pammeter. The teeth were thoroughly cleaned and any carious material carefully removed with a dental burr. Wherever "carious" enamel and dentin are mentioned in this report it is understood that the reference is to sound enamel and dentin from carious teeth. No

[1] This work was supported in part by the Carnegie Corporation of New York.

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carious material was included in any sample. The method of separation was the same as previously described (3) with the following exceptions: 1. The water jacketed centrifuge tube previously used to control the temperature was omitted. 2. A 50 cc. centrifuge tube was substituted for the 15 cc. tube used previously. 3. The same separation liquids were used with the exception that for the separation of enamel samples of density above 2.96 methylene iodide-tetrabromoethane solutions were used in place of iodoform-tetrabromoethane solutions.

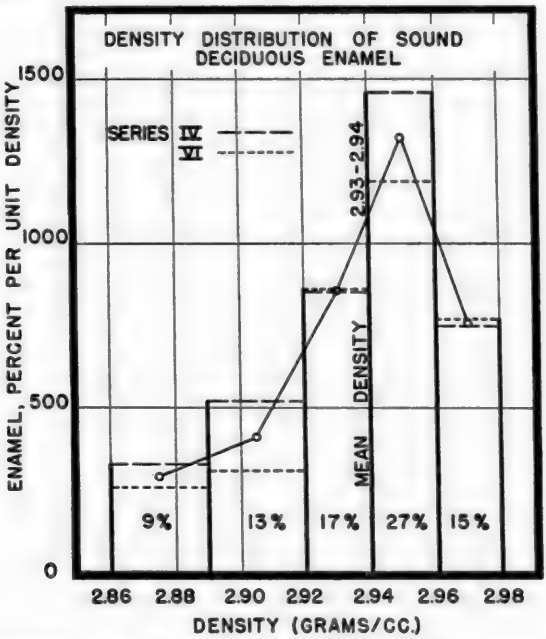

FIG. 1. Density distribution of sound deciduous enamel. Distribution curve has one maximum at 2.94–2.96. The percentages of the whole sample occurring in the various density fractions are written in the blocks. Mean density is 2.93–2.94.

RESULTS

In the block graphs representing the density distribution (*figs. 1–4*) the "per cent of enamel and dentin per unit density" was plotted as ordinate against the density as abscissa. In this way the heights of the various blocks are comparable regardless of the density limits; i.e., whether a given block represents a sample occurring in density limits differing by 0.02 or 0.03, the height of the block is the percentage of the original sample per unit density. The average per-

centage of the sample occurring in a given density fraction is written in that block. The average "per cent per unit density" within each density limit was calculated and these points are connected by the distribution curve. Table I contains the amounts in grams of enamel and dentin found in the various density fractions and the percentage losses accompanying each separation.

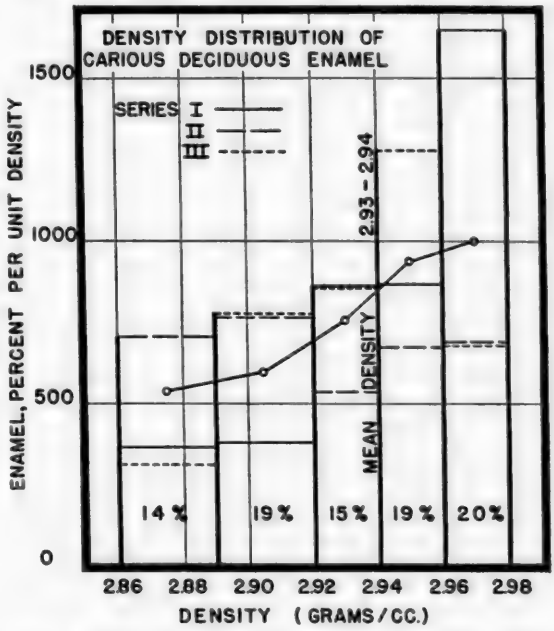

FIG. 2. Density distribution of carious deciduous enamel (sound enamel from carious teeth). Note general similarity to *fig. 1* except for higher average value at 2.96–2.98 due to series I.

Enamel. For both sound and carious dry enamel, the density distribution was measured between the limits of 2.86 and 2.98. For each, the mean density is between 2.93 and 2.94.

For sound dry enamel (*fig. 1*), the maximum of the curve lies between 2.94 and 2.96. The two samples check well although they are from different teeth. In series IV and VI the amounts of enamel of density below 2.86 are 10 and 26 per cent, respectively; of density above 2.98, 4 and 0.4 per cent, respectively.

For carious dry enamel (*fig. 2*) the maximum occurs between 2.96 and 2.98. Series I and II are duplicates and do not check closely; series III is from different teeth and checks with series II fairly well. In series I, II and III the amounts of enamel of density below 2.86 are 10, 13 and 8 per cent, respectively; of density above 2.98, 0.6, 4 and 5 per cent respectively.

TABLE 1

Amounts in grams of enamel and dentin found in the various density fractions and the percentage losses accompanying each separation

Part A. Enamel

DENSITY LIMITS	SOUND				CARIOUS*					
	Series IV		Series VI		Series I		Series II		Series III	
	Wt. in gm.	% loss	Wt. in gm.	% loss	Wt. in gm.	% loss	Wt. in gm.	% loss	Wt. in gm.	% loss
>2.86	0.8489	8	0.1282	14	0.2589	9	1.7312	3	0.4138	5
2.86–2.89	0.7161	6	0.0902	20	1.2591	4	1.4702	3	0.3919	5
2.89–2.92	0.6083	1	0.0647	4	0.9552	4	1.4245	18	0.3399	1
2.92–2.94	0.4930	9	0.0544	7	0.7938	2	0.7007	2	0.2536	8
2.94–2.96	0.3305	1	0.0372	7	0.5823	1	0.5156	3	0.1715	2
2.96–2.98	0.1270	0.4	0.0164	26	0.3820	1	0.2791	1	0.0812	22

Part B. Dentin

	Series IV		Series V		Series VI		Series I		Series II	
>2.10	0.3657	0.1	0.3069	2	0.0257	34	0.2273	4	0.9347	6
2.10–2.12	0.3276	3								
2.10–2.15	1.0503	13	1.2628	6	0.5932	5	1.4240	6	4.7984	6
2.15–2.18	0.2864	2	0.6335	1	0.2068	6	0.7221	2	2.3852	2
2.18–2.22	0.4335	4	1.1807	18	0.5343	1	1.1159	3	3.5382	5
2.22–2.26	0.5348	4	0.2148	3	0.3246	13	0.3584	25	0.9708	3

* "Carious" enamel and dentin are sound enamel and dentin from carious teeth.

The average loss for a single separation of enamel is 6.5 per cent of the sample (Table I), a value which compares favorably with the loss values previously reported (4).

Dentin. For both sound and carious dry dentin, the density distribution was measured between the limits of 2.10 and 2.26. For each, the mean density is between 2.18 and 2.19, and the maximum between 2.15 and 2.18.

The three series of sound dry dentin (*fig. 3*) are from different teeth, however the density distributions check fairly well. In series IV, V and VI the amounts of dentin of density below 2.10 are 5, 5 and 2 per cent respectively; of density above 2.26, 9, 9 and 30 per cent, respectively.

The two series used for carious dry dentin (*fig. 4*) are duplicates of the same pooled sample and check well. In series I and II the

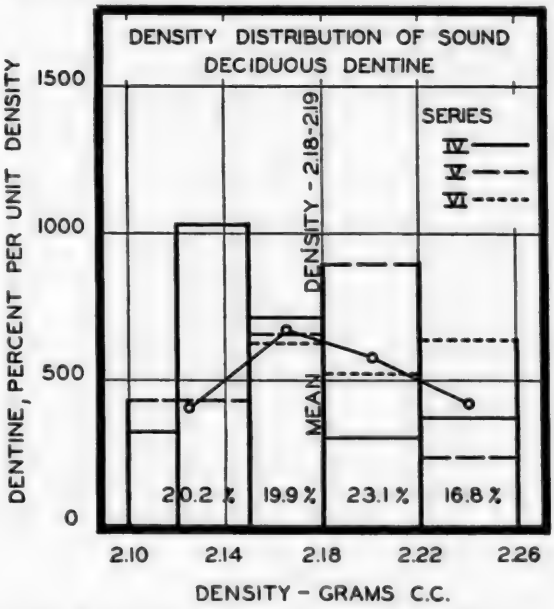

FIG. 3. Density distribution of sound deciduous dentin. Maximum at 2.15–2.18; mean—2.18–2.19).

amounts of dentin of density below 2.10 are 7 and 6 per cent, respectively; of density above 2.26, 12 and 15 per cent respectively.

The average loss for a single separation of dentin is 6.8 per cent of the sample (Table I). This value compares favorably with the loss values previously reported (4).

DISCUSSION

Enamel. Both sound and carious dry enamel approximate asymmetric distribution curves. In carious enamel the density distribu-

tion below 2.96 is closely similar to that of sound enamel. Above 2.96 the difference in "average per cent per unit density" is due to the very high value from series I. This difference may be explained by the hypothesis that a major portion of the enamel has a density of almost 2.96 and that small variations in experimental technique have produced a shift of the amounts of enamel separated into heavier and lighter fractions.

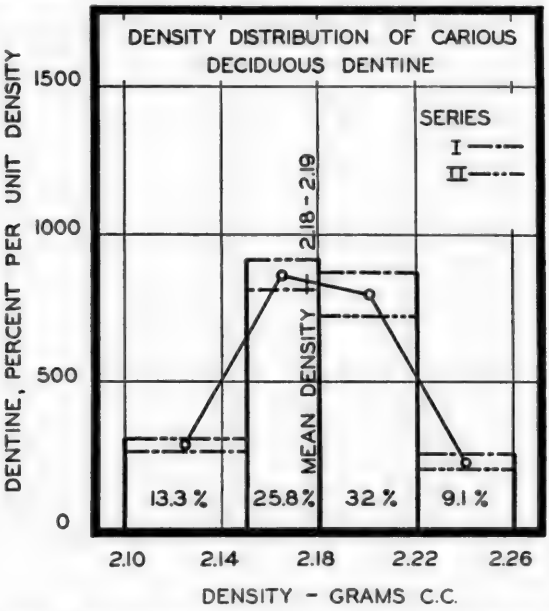

FIG. 4. Density distribution of carious deciduous dentin (Sound dentin from carious teeth). Note general similarity to *fig. 3* except somewhat sharper maximum.

Some support for this hypothesis is found in the relative amount of enamel in the 2.96–2.98 density fraction of Series II, a duplicate determination on the same pooled sample from which series I was obtained. In series II, 13 per cent of the enamel has a density 2.96–2.98 whereas 33 per cent was obtained in series I. In general, the density distributions for sound and carious deciduous enamel are nearly identical.

The dry enamel from deciduous teeth differs in density distribution from that of permanent teeth in four particulars. 1. The deciduous

enamel is more homogeneous than permanent enamel. Deciduous enamel has only one maximum (2.95) as compared with two for permanent enamel (2.91 and 2.97). 2. The deciduous enamel has a lower average density than that from permanent teeth. The mean density of deciduous enamel falls between 2.93–2.94; of permanent enamel between 2.95–2.96. The lower density of deciduous enamel is in line with (a) its lower inorganic content (93 per cent for deciduous enamel as compared to 96 per cent for permanent enamel (5); (b) its greater penetrability of dyes (6); and (c) its differences in birefringence (7). 3. Deciduous enamel contains less material of density greater than 2.98 than is found for permanent enamel; specifically, 3 per cent of deciduous enamel and 16 per cent of permanent enamel have a density greater than 2.98. 4. Deciduous enamel has more material of density less than 2.90 than is found for permanent enamel; specifically 22 per cent of deciduous enamel and 14 per cent of permanent enamel have a density less than 2.90.

To summarize, from the standpoint of density distribution, deciduous enamel, whether from sound or carious teeth, is a homogeneous tissue whose mean density is slightly less than that of permanent enamel but in general, deciduous and permanent enamel are surprisingly alike in densities.

Dentin. The density distributions of carious and sound dry dentin both approximate normal distribution curves, fall in the same density ranges and have the same mean density. The difference between density distributions of sound and carious dentin occurs in the relative amounts at given density intervals: sound dentin has higher values at 2.10–2.15 and at 2.22–2.26 than carious, but lower values in the intermediate ranges. Thus, 43 per cent of the sound dentin is found in the density limits 2.15–2.22 while 58 per cent of the carious dentin occurs in the same range. This difference may be due to the variations in root resorption in the teeth chosen for the various series. A pooled sample containing many teeth with marked root resorption would show relatively higher percentages of denser dentin since root dentin probably is of lower density (1). For example, in Series VI (*fig. 3*) a very small percentage of the whole sample (2 per cent) was found in the density range 2.10–2.15. The teeth which made up this sample were almost entirely lacking in roots.

The density distribution of dry deciduous dentin around the mean density of 2.18–2.19 differs from the distribution reported by Deakins, Boyd and Drain (1) principally in the value of the mean which they give as 1.91. This difference is not unexpected since their measurements on moist whole blocks would include approximately 10 to 15 per cent of water. It should be noted that in our calculations the amounts of dry dentin outside the known density limits, i.e., of density less than 2.10 and greater than 2.26, were necessarily omitted from the estimation of the mean density.

Density ranges for dry deciduous dentin are similar to those for dry permanent dentin, however there are certain differences. 1. The mean density of deciduous dentin is 2.18–2.19; of permanent dentin[3] is 2.14. The difference in mean density is not in line with the similarity of inorganic content. Deciduous dentin and permanent dentin have about the same inorganic percentage, viz., about 70 per cent (5). The apparent higher density of deciduous teeth is probably due to the root resorption which removes root dentin of lower density. Moreover the close physical similarity of deciduous and permanent dentin has been shown in the similarity of hardness values (8). 2. Deciduous dentin has more material of density less than 2.10 than has permanent dentin; specifically, 19 per cent of deciduous dentin and 5 per cent of permanent dentin has a density less than 2.10. 3. Deciduous dentin has about the same amount of material of density greater than 2.26 as has permanent dentin; specifically, 10 per cent of deciduous dentin and 15 per cent of permanent dentin has a density greater than 2.26.

To summarize, from the standpoint of density distribution deciduous dentin, whether from sound or carious teeth, is a homogeneous tissue whose density distribution is similar to that of permanent dentin.

SUMMARY

1. The density distribution curves of dry deciduous enamel and dentin were investigated for both sound and carious teeth by a centrifugal flotation method.

[3] It is interesting to note that in permanent dentin from carious teeth, a mean density of 2.19 has been previously reported (4).

2. Eighty-seven per cent of dry carious deciduous enamel and 81 per cent of dry sound deciduous enamel have a density between 2.86 and 2.98.

3. Dry deciduous enamel has only one maximum (2.94–2.96) in its density distribution curves.

4. Eighty per cent of both sound and carious dry deciduous dentin has a density between 2.10 and 2.26. The maxima in the distribution curves occur at 2.15–2.18.

5. In many respects, the density distributions of dry deciduous enamel and dentin are similar to those of the permanent tissues.

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DENDRITIC CELLS IN THE ALVEOLAR GINGIVA AND ENAMEL EPITHELIUM; DESTRUCTION OF THE ENAMEL EPITHELIUM BY PHAGOCITIC CELLS[1]

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I. DENDRITIC CELLS IN THE ALVEOLAR GINGIVA AND ENAMEL EPITHELIUM

Of a considerable number of investigators who have described the presence of dendritic cells in mucous membranes, but few have mentioned their presence in the oral and dental tissues. Adachi and Ramel recorded systematic investigations of normal mucous membrane pigmentation, such pigmentation being interpreted as functions of dendritic cells. Many references have been made to mouth pigmentation in dark races and in such pathologic conditions as Addison's disease, lymphogranuloma, hematoporphyrinuria, hyperthyroidism, parasitic melanoderma, primary anemia, tuberculosis, scleroderma, arsenical melanoderma and arsphenamine dermatitis. Strempel and Armuzzi, Reiche, Bonnet and Lebeuf, Jessner and others recorded pigmented macules in lichen planus. Silver and dopa reactions were first employed in the study of buccal mucous membrane by Ramel, who proved that normal tissue gives a dopa-positive reaction, and that the mechanism for pigment formation is the same as in the skin. The literature on the subject has been reviewed by Graupe, who also added his own observations.

The following is a report of a single case in which dendritic cells were observed in oral mucosa, and in the enamel epithelium as well; it is submitted because the cells were revealed by a special silver stain, which, as far as we can determine, has not been employed in the study

[1] Read at The 18th General Meeting of the International Association for Dental Research, Philadelphia, Pa., March 13-14, 1940. (*J. D. Res.* **19:** 292, 1940).

of dental tissues, and which has very interesting properties of revelation:

Post mortem examination of a white male child of 11 months, that had succumbed to pneumonia, revealed a number of pathological conditions, including several blood disorders as follows:

1. Congenital generalized osteosclerosis.
2. Markedly secondary anemia and purpura.
3. Bilateral confluent bronchopneumonia.
4. Chronic splenic tumor.
5. Hematuria.
6. Internal hydrocephalus.
7. Cortical atrophy of the brain.

The child had been cyanotic from birth, gave a history of congenital heart disease, loss of weight as a result of its refusal to nurse, and had suffered chronic constipation. No teeth had erupted.

A small cone containing the deciduous first incisor teeth, as well as the tooth buds of the permanent first incisors, was removed from the mandible. The tissue was fixed in 10 per cent formalin, decalcified in Custer's solution of formic acid and sodium citrate, sectioned in celloidin, and stained with hematoxylin and eosin. The teeth were found to have been poorly calcified and examination of the alveolar gingiva and the reduced enamel epithelium revealed an apparently normal histological picture.

A number of sections were stained with activated protargol in accordance with Bodian's method (1). This stain also revealed the poorly calcified condition of the teeth. In addition, the silver stain brought out structures not observed with the hematoxylin and eosin (*figs. 1* and *2*). In the alveolar gingival epithelium there were dendritic, nerve-like cells, with beaded, branching processes suggesting unipolar, bipolar, and multipolar neurones. The cell body varied in shape from globular to ovoid, rhomboid, polygonal, pyramidal, and star-shaped. The granular processes, resembling broken strings of beads, ran between the epithelial cells and extended in some cases from the cell body to the outer cornified layer and to the underlying connective tissue. Nuclei were not clearly distinguishable, although many cells suggested the presence of such a structure. The cells were large, darkly stained, and scattered through the gingival epithelium.

Only a very few were found in the basal layer, despite the fact that the cells were very numerous in most sections.

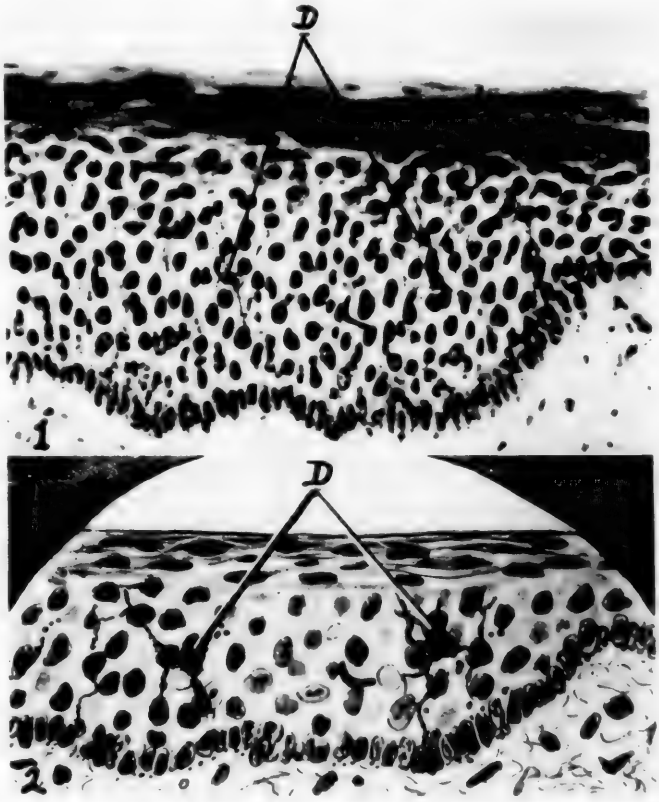

FIG. 1. Photomicrograph of section of aveolar gingiva (post mortem material), stained with activated protargol. Note cells (D) with granular, hair-like processes. Cells in outer epithelium.

FIG. 2. Drawing of section in *fig. 1*, showing dendritic cells (D) more clearly.

Some sections were subjected to the "dopa" reaction, according to Becker's (2) method, and the dendritic cells were found to stain dopa-positive. The cells observed in the gingival epithelium resembled melanoblasts, which were present in the skin.

Biopsy material was collected from the gingivae of 10 light-skinned

Caucasians and 10 dark-skinned Negroes. In the latter, the material was taken from pigmented areas in the gingiva. The material was treated exactly as was that taken from the child and stained with Bodian's activated protargol (*fig. 3*). It was possible to demonstrate the dendritic cells in this normal gingival epithelium, but they were fewer in number, and all those observed were found in the Mal-

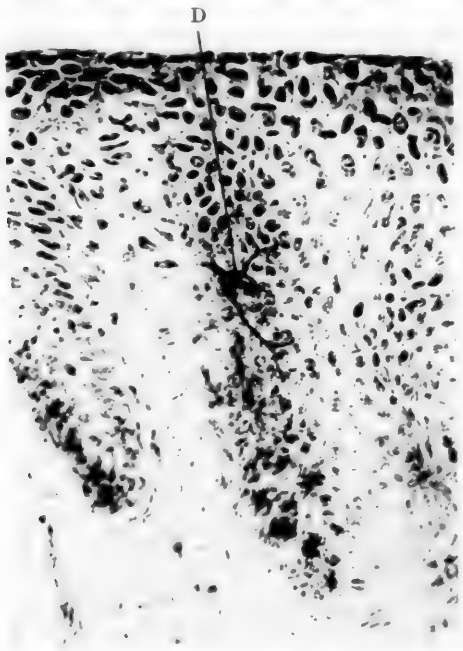

FIG. 3. Photomicrograph of section of alveolar gingiva (biopsy material), stained exactly as section in *fig. 1*. Note dendritic cells (D) with more darkly staining cell body and processes. Cell in Malpighian layer.

pighian layer. The cells observed in the normal tissue more closely resembled cells in the skin, and they differed slightly from the cells observed in the postmortem material in that they stained more darkly, were not as numerous, and were not scattered throughout the gingival epithelium, but instead, were limited to the Malpighian layer. Furthermore, it was even more difficult to discern nuclear bodies.

Examination of the reduced enamel epithelium (*figs. 4* and *5*) of the

tooth buds in the postmortem material of this child revealed the dendritic cells, but here they were fewer in number, and the processes

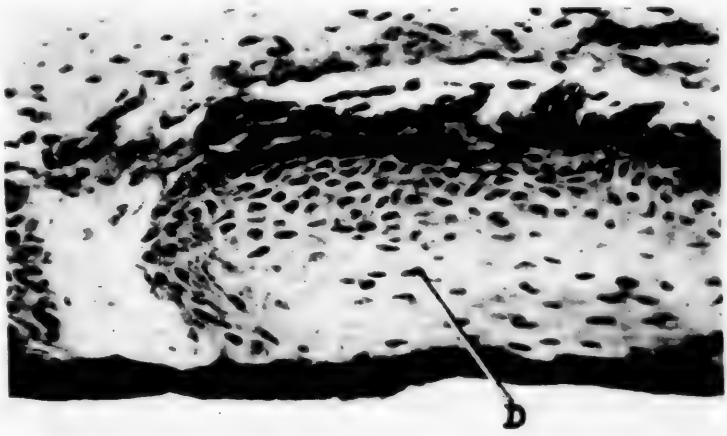

FIG. 4. Photomicrograph of section through the enamel organ of the deciduous tooth. Note pyramidal cell (D) with "tail." (Activated protargol stain.)

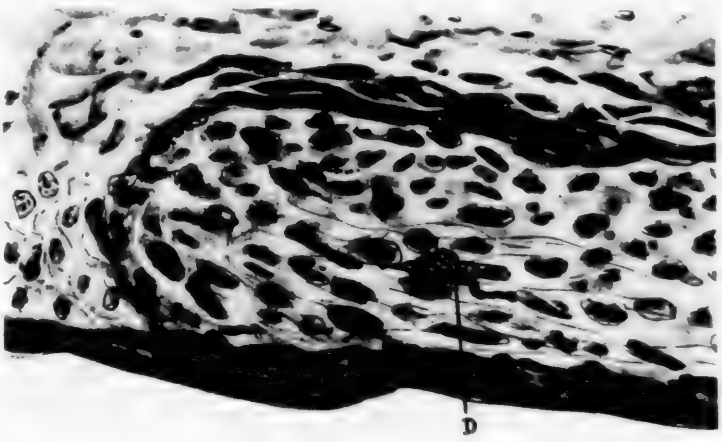

FIG. 5. Drawing of section in *fig. 4*

were shorter. Scattered through the enamel organ could be seen broken pieces of the granular processes.

In an examination of the dental literature and the texts available

to us, there was found no mention of these dendritic cells in the reduced enamel epithelium of the developing and erupting teeth. However, cells closely resembling the above description of the dendritic cells found in the gingiva and enamel organ are described in the buccal mucosa and interpreted as melanoblasts by Becker (2), who cites references. Laidlaw and Cahn (5) describe melanoblasts in the gingivae. The principal difference between Becker's melanoblasts, as well as those of Laidlaw and Cahn, and those described by us seems to be in their location. These investigators mention the cells as confined to the Malpighian layer of the mucosa, with an occasional individual seen farther out in the epithelium. The cells observed by us in the gingival epithelium and the enamel organ are very numerous, and their greatest distribution is among the outer epithelial cells, with only an occasional cell seen in the basal layer.

Edwards and Duntley (3) and Becker (2) suggest that pigment cells may have some bearing in the diagnosis of blood dyscrasias and other diseases. The dendritic cells observed in the gingival epithelium suggest melanoblasts, or pigment cells, and the fact that they were so numerous in the tissue of the child, which give a history of anemia and other diseases, and comparatively few in the tissue of normal individuals, suggests the possibility that perhaps these cells are an indicator of some pathological sequence. Laidlaw and Cahn, referring to the observations and reports of Treves, Roy, Stewart and Phillips, New and Hansell and others, suggest that the presence of melanoblasts in the gingivae "offers a rational explanation of the growth of malignant melanoma in the gum" and say "Where pigmented areas exist in the gum, it is advisable to keep them free from irritation of dentures and oral sepsis."

II. DESTRUCTION OF THE ENAMEL EPITHELIUM BY PHAGOCITIC CELLS

In the same material in which the dendritic cells were observed in the gingival tissue and in the enamel epithelium another unusual phenomenon was observed, which warrants description. Examination of the reduced enamel epithelium in a number of sections (*fig. 6*) stained by the activated protargol, revealed large phagocytic cells in the act of engulfing the epithelial cells, thus destroying them and leaving large hyalinized areas. Our sources of reference make no

mention of this phenomenon, but indicate, rather, that there is a gradual cytomorphosis of the epithelial cells of the enamel organ. Schour (4) suggests in this connection, that there "must be some method whereby the epithelium is disposed of and the degenerated cells removed," and he observed in some hypophysectomized animals that "the labial alveolar periosteum showed clusters of degenerated epithelial cells which apparently came from the enamel epithelium." He suggests that some of these clusters may have been large phagocytes, which incorporated the epithelial debris.

FIG. 6. Photomicrograph of section through the enamel organ of deciduous tooth, showing phagocytic (P) action.

The observation of dendritic cells in the alveolar gingiva and in the reduced enamel epithelium, and also of the phagocytic cells in the enamel organ are of interest, because they are present in the dental tissues and were disclosed by special stain technic. Further investigation of the dendritic cells may disclose a connection with blood dyscrasias or other diseases, as well as malignant tumors. Observation of phagocytic activity in the enamel organ reveals an interesting phenomenon apparently not hitherto afforded very much attention. A greater employment of special stains in the study of dental tissue may reveal phenomena not in evidence when ordinary stains are used.

SUMMARY

1. Sections of material taken from the mandible of an 11 month white male child at post mortem were stained with hematoxylin and eosin, and with Bodian's activated protargol stain.

2. Sections stained with hematoxylin and eosin revealed nothing unusual. Those stained with the silver stain revealed the presence of dendritic cells throughout the gingival epithelium and in the enamel organ of the erupting teeth. The cells observed in this tissue differed from those described in the literature chiefly in the fact that they were located principally in the outer epithelial cells, while those reported in the literature occurred in the Malpighian layer. The cells described in this report gave a dopa-positive reaction, and seemed to take a lighter stain than similar cells observed in normal tissue obtained in biopsies from light-skinned Caucasians and dark-skinned Negroes.

3. In some of the sections stained with Bodian's activated protargol the enamel organ contained large phagocytic cells in the act of engulfing epithelial cells, and producing large hyalinized areas.

4. There may be some connection between the presence of dendritic cells and blood dyscrasias or other diseases. The presence of dendritic cells may offer an explanation for malignant melanoma.

5. Bodian's activated protargol stain might reveal other interesting phenomena, if employed in the study of dental tissues.

Much credit is due Miss R. A. Penfield, of the Department of Anatomy, for introduction to Bodian's activated protargol stain, and to Dr. R. A. Knouf, of the same department, for advice and suggestions regarding identification of the cells herein described. We are grateful also for generous aid in the interpretation of these structures to Dr. R. Kronfeld, Dr. I. Schour, Dr. B. Orban, Dr. S. W. Chase, and Dr. L. R. Cahn.

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